REMARKS/ARGUMENTS

Claims 1-11, 19, and 26-35 are pending in the application, and were rejected. In response thereto, Applicant has amended Claims 1, 3-4, 11, 19, and 33-34, and added new Claims 37-38. Claims 32 and 35 have been cancelled. As amended, the claimed invention is direct to a method for treating chronic wounds using a microbial cellulose dressing "consisting essentially of" 1.5 to 4.5 wt.% microbial cellulose and water. Further, Claim 19 recites a method of preparing such a dressing in which an intermediate pellicle has a cellulose to water ratio in the range of about 1:100 to about 1:500 – which corresponds to 1 to 0.2 wt.% cellulose, and is then dried to form a microbial cellulose dressing "consisting essentially of" 1.5 to 4.5 wt.% microbial cellulose and water. Further, both independent claims recite an absorption ability and donation ability of greater than 75% of the liquid weight. Further, new Claim 36 is directed the processing conditions for the purification step. Support for this new claim is found in Paragraph 0040 of the published application.

A. The Ring '400 Patent

In paragraph 3, the Examiner rejected Claims 1-11, 19, and 26-35 under 35 U.S.C. § 102(b) as being anticipated by Ring, U.S. Patent No. 4,588,400 ("the Ring '400 Patent"). Applicant respectfully traverses the rejection in light of the amended claims.

It is well settled that "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." See Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 U.S.P.Q.2d 1051, 1053 (Fed. Cir. 1987). Further, "[w]hen the prior art discloses a range which touches or overlaps the claimed range, but no specific examples falling within the claimed range are disclosed, a case by

case determination must be made as to anticipation. In order to anticipate the claims, the claimed subject matter must be disclosed in the reference with sufficient specificity to constitute an anticipation under the statute." See MPEP § 2131.03. What constitutes a "sufficient specificity" is fact dependent. In particular, if the claims are directed to a narrow range, and the reference teaches a broad range, one can conclude that the narrow range is not disclosed with "sufficient specificity" to constitute an anticipation of the claims. See, e.g., Atofina v. Great Lakes Chem. Corp, 441 F.3d 991, 999, 78 U.S.P.Q.2d 1417, 1423 (Fed. Cir. 2006) (holding that a reference temperature range of 100-500 °C did not describe the claimed range of 330-450 °C with sufficient specificity to be anticipatory). Further, any evidence of unexpected results within the narrow range may also render the claims unobvious.

In this case, the Ring '400 Patent contemplates liquid loaded pads having a very wide range of cellulose contents and a very wide range of liquid types in the liquid loaded pad. As FIG. 1 of the present application shows, liquid loaded pads having very low (e.g., 0.7 wt.%) and very high (e.g., 20 wt.%) cellulose contents are unable to both donate and absorb liquid essential for treating non-responsive chronic wounds as claimed. As discussed more fully below, none of the examples in the Ring '400 Patent fall within the claimed cellulose content range and the claimed liquid type (water). As such, Applicant respectfully submits that the claimed invention is not anticipated by the Ring '400 Patent.

Example 1 of the Ring '400 Patent teaches a saturated cellulose pellicle comprising 40 g/M^2 cellulose and 3600 g/M^2 water. This corresponds to a cellulose content of 1.1 wt.% cellulose.[1] Example 1 does not anticipate the claimed invention for at least three reasons. First, the cellulose content of Example 1 is outside the claimed range of 1.5 to 4.5 wt.% cellulose.

[1] The calculation is (40 g/M^2)/(40 g/M^2 + 3600 g/M^2) or 1.1 wt.%.

Second, FIG. 1 of the present application shows that such low cellulose values (near 1%) are unable to both donate and absorb liquid as claimed. Third, the Ring '400 Patent provides no evidence that the liquid loaded pads may successfully treat non-responsive chronic wounds. Thus, Applicant respectfully suggests that the claimed invention is not anticipated by Example 1 of the Ring '400 Patent.

Example 2 of the Ring '400 Patent teaches a water loaded pellicle having a water to cellulose ratio of 8:1, which corresponds to 11.1 wt.% cellulose. This is well outside the claimed range of 1.5 to 4.5 wt.% cellulose. Example 2 prophetically describes a water-loaded pellicle having a liquid to cellulose content ranging from 2:1 to 20:1 that "may be prepared" (col. 5, line 38). This corresponds to 33% to 4.8% wt.% cellulose. Again, this is outside the claimed range. Further, there is no teaching that such a dressing can be used for the effective treatment of a chronic wound as claimed.

Examples 3-8 of the Ring '400 Patent either recite processing conditions that make it difficult to calculate the cellulose content, have cellulose contents clearly outside the claimed range, or include chemicals (such as glycerol, polyethylene glycol, or petrolatum) that negatively affect performance of the product if used as a chronic wound dressing.

More specifically, Example 3 of the Ring '400 Patent teaches a sheet according to Example 2 (i.e., a water-to-cellulose ratio of 8:1 or 11 wt.% cellulose) immersed in water, water/glycerol, or saline, in which "about 70% of the original liquid content" was recovered. Thus, the liquid content in Example 3 is actually decreased by 30% so that the ratio is 5.6 to 1 (70% x 8:1), which corresponds to 15 wt.% cellulose. Again, this is outside the claimed range such that Example 3 does not anticipate the claimed invention.

Example 4 of the Ring '400 Patent teaches a sheet material reconstituted with glycerol or polyethylene glycol ("PEG") to obtain a liquid content of 2000 g/M^2 liquid and 40 g/M^2 cellulose (from Example 1) or a liquid to cellulose ratio of 50:1. Thus, the resulting material had a cellulose content of 2 wt.% and water and glycerol or PEG. Importantly, the claimed invention is limited to a wound dressing "consisting essentially" of microbial cellulose and water. As set forth in the Damien Declaration under § 1.132 (attached as Exhibit F), Applicant has conducted experiments to show that the claimed invention has donative/absorptive properties superior to that of Examples 4, 7, and 8 of the Ring '400 Patent.

Example 5 of the Ring '400 Patent teaches air drying the pellicle and then immersing the sheet in glycerol where it regains about 5% of its original liquid content. Five percent of 3600 g/M^2 is 180 g/M^2. Thus, the resulting material comprised 40 g/M^2 cellulose and 180 g/M^2 glycerol or about 18 wt.% cellulose. Thus, the claimed invention is not anticipated by Example 5 of the Ring '400 Patent because the cellulose content is well outside the claimed range.

Example 6 of the Ring '400 Patent teaches reconstituting the sheet material with polyvinylpyrolidone ("PVP") to about 70% of its original liquid content. The pellicle was then allowed to air dry to about 50% of its reconstituted weight. The pellicle was then exposed to an electron beam to cross link the PVP to form a gel within the pellicle. Thus, Example 6 of the Ring '400 Patent does not anticipate the claimed invention because it teaches a PVP cross-linked "gel" in the dressing and does not "consist essentially of" water and microbial cellulose as claimed.

Example 7 of the Ring' 400 patent teaches reconstituting the sheet material with 1% silver sulfadiazine ("SSD") ointment. The liquid content increased to about 1000 g/M^2. Thus, the resulting material comprised 40 g/M^2 cellulose and 1000 g/M^2 liquid (water and SSD) or

about 3.8% cellulose. As set forth in the Damien Declaration, Applicant has conducted experiments to show that the claimed invention has donative/absorptive properties superior to that of Examples 4, 7, and 8 of the Ring '400 Patent.

Example 8 of the Ring '400 Patent teaches reconstituting the sheet material with water to 2000 g/M^2 and then immersing the pellicle in petrolatum. Thus, the resulting material comprised 40 g/M^2 cellulose and 2000 g/M^2 liquid (water and petrolatum) or about 2% cellulose. The resulting product was a petroleum-coated dressing having a water core. As set forth in the Damien Declaration, Applicant has conducted experiments to show that the claimed invention has donative/absorptive properties superior to that of Examples 4, 7, and 8 of the Ring '400 Patent.

In the Office Action, the Examiner relies upon the intermediate 2% product in Example 8 as a basis for rejecting Applicant's claims. Applicant respectfully submits that Example 8 actually teaches away from the amended "method of treatment" claims of the present invention. That is, Example 8 teaches only that the final petrolatum-coated product should be applied as a treatment for acute wounds, not the intermediate product. In contrast, the present invention is directed to a method of treating chronic wounds with a microbial cellulose dressing consisting essentially of from about 1.5% to about 4.5% cellulose and water. Moreover, in order to clarify that the intermediate product is excluded from the claim scope, Applicant has amended Claim 1 to clarify that the treatment involves a "kit" the microbial cellulose dressing in a moisture-proof package. In addition, new Claims 37-38 recite the packaging.

In short, none of the examples in the Ring '400 Patent disclose a method of treating chronic wounds with a microbial cellulose dressing consisting essentially of 1.5 to 4.5 wt.% cellulose that is capable of both absorbing and donating liquid in the amount claimed. In

dressings with less than 1.5 wt.% microbial cellulose, the dressing fails to absorb significant amounts of exudates from a chronic wound. With amounts more than 4.5 wt.% microbial cellulose, the dressing fails to hydrate the wound bed adequately. The dressings in the examples describe only examples which either absorb (highest cellulose range) or hydrate (lowest cellulose range) – but not both. See column 3, lines 29-31 ("either supply moisture to the wound or absorb exudate"). The claimed dressing is capable of donating about 75% to 95% of its liquid weight and is also is capable of absorbing between about 35% to 75% of its liquid weight where the wound has exudates. See FIG. 1. These properties were not recognized in the prior art and are critical in the treatment of chronic wounds.

Equally important, Applicant respectfully submits that the claimed method of treating a specific type of wound – non-responsive chronic wounds in humans – is not anticipated by the Ring '400 Patent. The Ring '400 Patent only mentions the possibility of treating ulcers hypothetically with liquid loaded pads having an occlusive film. See col. 9, lines 24-50 ("A dressing ....may be applied to such an ulcer..."). Yet, the Ring '400 Patent does not specify what type of ulcers are contemplated. Nor is there any evidence of healing success in treatment of non-responsive chronic wounds over a long period of time. The only biological data disclosed in the Ring' 400 Patent deals with the use of the liquid loaded pad from Example 4 (which contained PEG or glycerol) in guinea pig studies in which the full thickness of skin in the dorsal area was surgically removed. See column 7, line 60 to column 8, line 4. Further, the Ring '400 Patent only investigated treatment for 8 days. Clearly, this type of "surgical wound" is an acute wound and not a chronic wound as claimed. A chronic wound is a non-responsive wound that has failed to proceed through an orderly and timely process to produce anatomic and functional integrity, or one that has proceeded through the repair process without establishing a sustained

anatomic and functional result. See Lazarus et al., *Definitions and Guidelines for Assessment of Wounds and Evaluation of Healing*, Arch Dermatol. 130:489-493, at pg. 490 (1994) (attached as Exhibit A). For these additional reasons, Applicant respectfully submits that the claimed invention is not anticipated by the Ring '400 Patent.

In addition, in Davis et al., *Wound environment: implications from research studies for healing and infection* in: Krasner DL, Rodehaver GT, Sibbald RG eds. CHRONIC WOUND CARE: A CLINICAL SOURCE BOOK FOR HEALTHCARE PROFESSIONALS (4th ed. Malvern, Pa: HMP Communications), at pg. 205-213 (2007) (attached as Exhibit B) the authors recognized that "small mammal[s] (e.g., mouse, rabbit, guinea pig)... have striking differences to humans [since] they tend to have dense fur with relatively thin epidermal and dermal layers. Additionally, they heal mostly by wound contraction instead of re-epithelization." See pg. 205 line 14. Therefore, treating non-responsive chronic wounds in humans is not anticipated by the Ring '400 Patent, which contains only animal data for acute surgical wounds.

The literature also shows that different kinds of wounds require different kinds of dressings depending on their history and development. See Broussard, *Dressing Decisions* in: Krasner DL, Rodehaver GT, Sibbald RG eds. CHRONIC WOUND CARE: A CLINICAL SOURCE BOOK FOR HEALTHCARE PROFESSIONALS (4th ed. Malvern, Pa: HMP Communications), at pg. 249-262 (2007) (attached as Exhibit C). Therefore, Applicant respectfully submits that it is not obvious to apply dressings that the prior art teaches may be used on acute wounds over a relatively short period of time on non-responsive chronic wounds as in the present invention.

In short, Applicant respectfully submits that one skilled in the art would not equate the treatment of 8-day-old "acute wound" of the Ring '400 Patent with the treatment of non-responsive chronic wounds as claimed. Because the claimed invention is able to both absorb and

donate more than 75% of its weight of liquid, improved healing of chronic wounds is possible compared to that found in the prior art. For this additional reason, Applicant respectfully submits that the claimed invention is not anticipated by the Ring '400 Patent.

In the present invention, the microbial cellulose dressings having 1.5 to 4.5 wt.% cellulose were used to treat non-responsive chronic wounds. This had not been done before such that there is no anticipation of the claimed invention. The specification states in part:

> Example 4
>
> Human Clinical Effectiveness Testing in Treating Chronic Wounds
>
> [0057] The objective of the human clinical testing was to assess the effectiveness of the cellulose wound dressing in treating various types of chronic wounds. A total of 29 patients with 31 various types of chronic wounds were involved in the study. The patients were treated with the cellulose wound dressing after passing the inclusion criteria outlined in the study protocol approved by an institutional review board (IRB). The cellulose wound dressing treatment was implemented for eight weeks or until the wound healed. Weekly wound observations were conducted. After the observations were recorded the dressings were changed. Both wound condition and size were recorded during the weekly visits and the study was terminated after the wounds healed or eight weeks of treatment.
>
> [0058] The results of the human study can be divided into three notable indications based on the performance of the cellulose wound dressing. The cellulose wound dressing exhibited strength in the removal of slough necrosis in deep pressure ulcers. Application of the cellulose wound dressing reduced the hypergranulation tissue down to the level of the surrounding epithelium in two wound presented with the problem. The third and most interesting response to the cellulose wound dressing was observed during the treatment of venous leg ulcers, particularly those with full thickness tissue involvement. The results showed that out of thirteen (13) venous leg ulcers (two partial thickness and eleven full thickness wounds), seven (54%) were completely healed and the remainder (46%) showed improvement during the course of the eight-week study.

See Paragraph 0057-0058 (emphasis added). This clinical study was further detailed in Example 5 (Paragraphs 0059 to 0072) and Brown-Etris et al., *Evaluation of XCell Wound Dressing on Wound Healing of Pressure Ulcers* (2003) (attached as Exhibit D) and Brown-Etris et al.,

Evaluation of XCell Wound Dressing on Wound Healing of Venous Stasis Ulcers (2003) (attached as Exhibit E).

With respect to pressure ulcers, FIGs. 6-15 of Exhibit D describe how treatments prior to the use of XCell® wound care dressing included mechanical, chemical, and autolytic debridement and use of other wound dressings. Therefore, the success of XCell® (14 of 16 evaluable wounds; Table 1) in showing healing or improvement over the eight-week treatment was truly unexpected and further demonstrates that success in medical arts, especially in wound healing, is not as predictable as in mechanical arts.

Similarly, with respect to venous ulcers, FIGs. 4-6 of Exhibit E show the "[h]ealing of a venous ulcer [that was] classified as a non-responsive wound that had lasted one to three months. Previous treatments included mechanical debridement and use of other wound dressings. Over the study, the wounds continued to heal [...] to being fully healed at six weeks." Also, FIGs. 7-9 and FIGs. 10-11 demonstrate the unexpected success of XCell® quite strongly.

In sum, this evidence shows that the claimed invention (treatment of chronic wounds) was not anticipated by the cited prior art.

In view of the foregoing amendments and remarks, it is respectfully submitted that the claims are now in condition for allowance and eventual issuance. Such action is respectfully requested. Should the Examiner have any further questions or comments which need be addressed in order to obtain allowance, please contact the undersigned attorney at the number listed below.

Acknowledgement of receipt is respectfully requested.

Respectfully submitted,

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REVIEW ARTICLE

Definitions and Guidelines for Assessment of Wounds and Evaluation of Healing

Gerald S. Lazarus, MD; Diane M. Cooper, PhD, RN; David R. Knighton, MD; David J. Margolis, MD; Roger E. Pecoraro, MD†; George Rodeheaver, PhD; Martin C. Robson, MD

Background: Chronic wounds represent a worldwide problem. For laboratory and clinical research to adequately address this problem, a common language needs to exist.

Observation: This language should include a system of wound classification, a lexicon of wound descriptors, and a description of the processes that are likely to affect wound healing and wound healing end points.

Conclusions: The report that follows defines wound, acute wound, chronic wound, healing and forms of healing, wound assessment, wound extent, wound burden, and wound severity. The utility of these definitions is demonstrated as they relate to the healing of a skin wound, but these definitions are broadly applicable to all wounds.

(Arch Dermatol. 1994;130:489-493)

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†Deceased.

FEW STATISTICAL trends over the past several decades have been as consistent as those related to the increasing longevity of the American population. Though such a change appears salutary, it is not without consequences. One of the most obvious effects is the emergence of a growing segment of the population with chronic health care problems. Among the costlier sequelae of chronicity is the presence of a large number of individuals with indolent or chronic wounds. In addition to the emotional costs associated with the presence of a nonhealing sore is the escalating financial burden of the care of these wounds to patients, to families, and to society.

Pressure ulcers, or decubitus ulcers, are examples of such chronic wounds; there is an estimated 3% to 5% incidence rate in hospitalized patients.[1-4] The incidence increases to 25% to 85% in patients with spinal cord injuries.[5] Assuming that 5% of the approximately one million Americans hospitalized yearly will develop pressure sores, and using the 1977 estimate of $15 000 for cost of care per patient,[5] the total cost of treatment is a staggering $750 000 000 per year, not accounting for inflation.[1] Similar data are available for other chronic wounds.

More rapid healing of a chronic wound is significant because it could result in decreased hospitalization and earlier return of the patient to daily functions. Institutional care of such chronic wounds costs approximately $1000 per day. Because patients are increasingly cared for outside of hospitals, evaluation of wounds, availability of wound care supplies, and consistency of care vary enormously.

The consequence is that many chronic wounds last far longer than necessary. Some wounds never heal and these may indirectly be responsible for patients' deaths. As more care is rendered in the home, the need for therapies aimed at restoring and maintaining structural integrity increases.

Market expenditure of over $7 billion dollars worldwide has been projected for provision of such therapies. However, potential savings of $11 billion in health care costs have also been projected. With this background, it has become clear that confusion about wounds and healing has led to divergent initiatives and less productive approaches.[6] The Wound Healing Society, Richmond, Va, believes that definitions and guidelines for assessment of wounds and evaluation of healing are necessary to relieve this confusion.

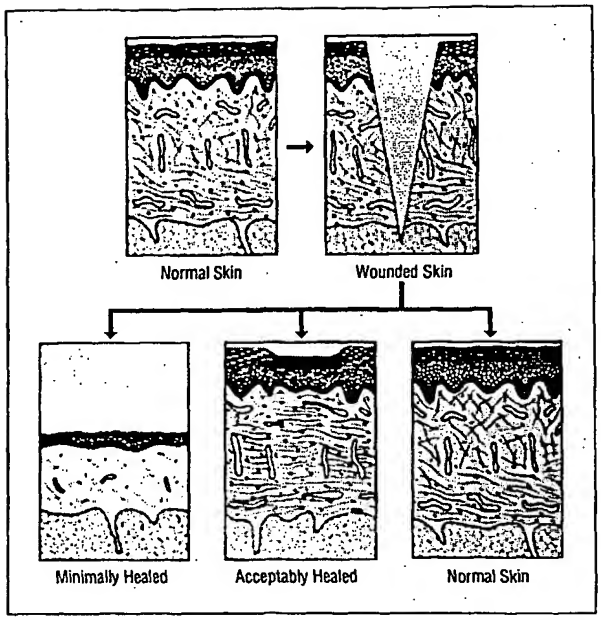

Figure 1. *A pictorial representation of prototypic forms of wound healing. An ideally healed wound results in a return to normal anatomic function, structure, and appearance. A minimally healed wound results in the restoration of anatomic continuity but without a sustained functional result. An acceptably healed wound is characterized by restoration of sustained functional and anatomic continuity.*

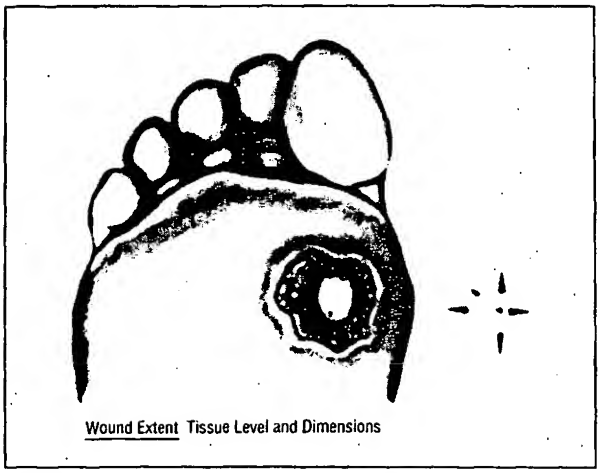

Figure 2. *Wound extent is based on the tissue level involved and the wound dimensions. The wound extent will change during wound healing and needs to be monitored.*

The purpose of this article is to initiate the creation of a common language defining a wound, healing, and the factors and processes that are important for wound healing. This language should include a system of wound classification, a lexicon of wound descriptors, and description of the processes that are likely to affect wound healing and wound healing end points. A consensus on terminology among parties interested in wound repair would greatly facilitate the ability of workers in this field to advance knowledge. The proposed definitions and guidelines are not intended as a dogmatic statement but rather as a thoughtfully prepared foundation for future discussions that will accommodate modifications over time. Although the impetus for embarking on this task was to provide basic definitions and guidelines for individuals doing research in wounds and healing, this approach will be of parallel value to clinicians, caregivers, regulators, and payers.

DEFINITIONS

A wound is a disruption of normal anatomic structure and function. Wounds result from pathologic processes beginning internally or externally to the involved organ(s). Acute wounds normally proceed through an orderly and timely reparative process that results in sustained restoration of anatomic and functional integrity. Chronic wounds have failed to proceed through an orderly and timely process to produce anatomic and functional integrity, or proceeded through the repair process without establishing a sustained anatomic and functional result. Orderliness refers to a sequence of biological events including the following: control of infection, resolution of inflammation, angiogenesis, regeneration of a functional connective tissue matrix, contraction, resurfacing, differentiation, and remodeling. Timeliness is relative, and it is determined by the nature and degree of the pathologic process, the status of the host, and the environment. The expectation of the length of time to wound repair must be clearly specified when distinguishing between an acute and chronic wound. Simply stated, wounds may be classified as those that repair themselves or can be repaired in an orderly and timely process (acute wounds) and those that do not (chronic wounds).

Healing is a complex dynamic process that results in the restoration of anatomic continuity and function. This usually involves the orderly sequence of biologic events listed previously. Healed wounds constitute a spectrum of repair and they must be defined and specified (**Figure 1**). An ideally healed wound is one that has returned to normal anatomic structure, function, and appearance. A minimally healed wound is characterized by the restoration of anatomic continuity, but without a sustained functional result and hence the wound may recur. Between these two extremes, an acceptably healed wound is characterized by restoration of sustained functional and anatomic continuity.

ASSESSMENT OF THE WOUND

Assessment of a wound in the environment in which it occurs is essential for diagnosis, treatment, management, and study. No wound can be assessed in isolation from the patient or his or her environment. Thus, complete wound assessment must include the extent of the wound, associated attributes of the wound, host factors that influence wound status, and environmental factors that impact on optimum wound management. We pro-

Table 1. Approaches Used to Determine Wound Extent

Parameter	Noninvasive	Invasive
Level	Visual, ultrasound, roentgenogram	Surgical debridement, biopsy
Perimeter/area	Linear measurement, acetate tracing, planimetry	
Volume	Linear measurement, Kundin gauge, stereophotometry, magnetic resonance imaging, ultrasound	Liquid capacity, molds

Table 2. History and Physical Examination Findings Important in Defining Wound Attributes

Wound	Periwound
History	
Location	Spontaneous pain
Duration	Induced pain
Spontaneous pain	
Induced pain	
Positional pain	
Prior wound manipulation	
Exudate	
Odor	
Physical Examination	
Location	Erythema
Color, elevated/dependent	Induration
Odor	Edema
Fibrin	Lymphangitis
Necrosis	Callus
Undermining	Joint abnormalities
Tunnel/sinus formation	Capillary refill
Exposed tissues	Hair distribution
Instrument probe	Exposed tissue
	Function and status of surrounding organs

pose that the following terms and relationships are useful in the assessment of wounds. This relationship can be defined by the following: extent *alpha* tissue level, wound dimensions; wound burden *alpha* extent, attributes; and wound severity *alpha* wound burden, host, environment.

Assessment of any wound should begin with the extent of the wound (**Figure 2**). Because extent of a wound is a dynamic process, it requires repeated systematic assessment. The total wound extent is based on the tissue level involved and the wound dimensions. The determination of extent of a wound can include noninvasive and invasive technologies (**Table 1**). The noninvasive assessment of extent includes perimeter, maximum dimensions of length and width, surface area, volume, amount of undermining, and determination of tissue viability. Invasive methods may be necessary to quantify the extent of a wound. The tissue levels of the wound must be defined from its surface to its depth and may vary depending on the organs involved. The total wound extent should be determined by the integration of the maximal amount of available data.

A wound can be further described by various attributes, which include the following: duration, blood flow, oxygen, infection, edema, inflammation, repetitive trauma and/or insult, innervation, wound metabolism, nutrition, prior wound manipulation, and systemic factors. These attributes are clues to the cause, pathophysiology, and status of the wound. The first step is a complete and careful history and physical examination. **Table 2** presents important aspects of the history and physical examination that are helpful in defining attributes. These should be carefully monitored and documented. There are a number of noninvasive and invasive technologies that can assist in quantifying attributes (**Table 3**).

Ultimately, wounds should be assessed by their effect on the host. These factors are defined by the terms wound burden and wound severity. Wound burden is a function of the extent of the wound and its attributes

Table 3. Inventory of Technologies Used to Evaluate Wound Attributes*

Attribute	Noninvasive	Invasive
Blood flow/oxygenation	Thermography, infrared recorder, transcutaneous Po_2, transcutaneous Pco_2, laser Doppler, Doppler waveform, ankle brachial index, pulse volume recording, toe pressure, duplex waveform, magnetic resonance imaging flow profile, isotope washout, NAD/NADH fluorometry	Arteriography, subcutaneous Po_2, venography, lymphangiogram, fluorometry
Infection	Roentgenogram, bone scan, magnetic resonance imaging, indium 111 scan	Biopsy for culture, probe to bone, biopsy for histologic examination, swab culture
Edema	Organ/extremity circumference, venous plethysmography, duplex venous imaging, Doppler venous examination	Venography, lymphangiography, venous pressure
Excessive inflammation	Thermography, laser Doppler	Biopsy for histologic examination
Repetitive insult	Computer pressure profile, thermography	
Innervation	Semmes-Weinstein filaments, two-point testing, vibration testing, sweat test	Nerve conduction, electromyography

A number of these tests are limited in their availability. The relative merits of these technologies may still need to be evaluated.

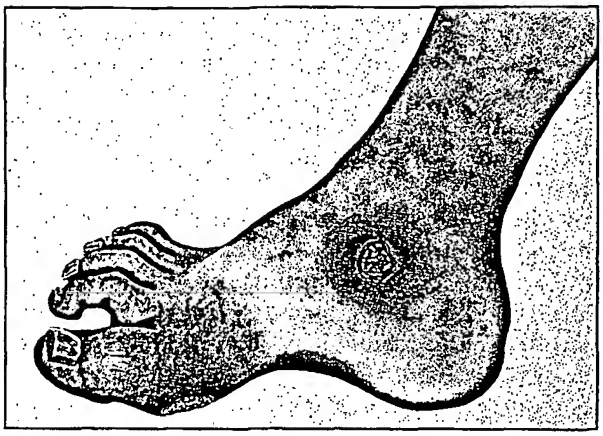

Figure 3. *Wound burden is a function of the extent of the wound and its attributes. Wound attributes are listed as follows: duration, edema, infection, inflammation, innervation, nutrition, oxygenation, trauma, and wound metabolism.*

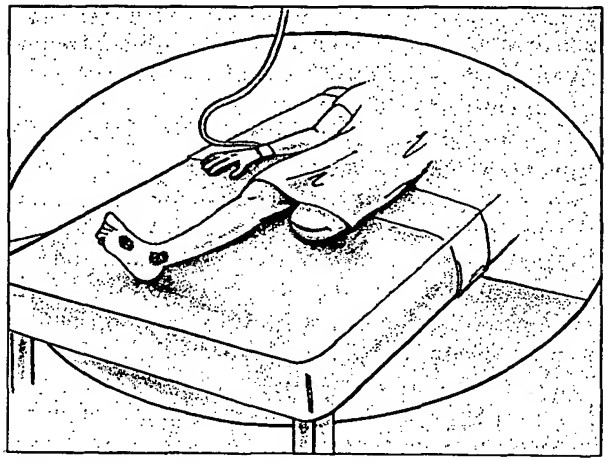

Figure 4. *Wound severity reflects wound burden, host factors, and environment. Wound severity changes during wound healing, but also when changes are made to the host and the environment.*

(**Figure 3**). Wound severity reflects wound burden, host factors, and environment (**Figure 4**). These characteristics can change during healing.

The status of the patient is essential to understanding the cause as well as evaluating the impact of systemic factors on the wound. In addition, there are environmental factors that influence the access to, and the quality of, care required to optimize the potential for wound repair. These factors include demographics, systemic agents that affect wound repair, and systemic disorders (**Table 4**).

EVALUATION OF HEALING

Evaluation of healing requires the analysis of qualitative and quantitative wound assessments. The simplest method to evaluate the outcome of healing is to examine the healed wound and determine if it is minimally, acceptably, or ideally healed. This may be accomplished by history and physical examination alone, but it may require objective

Table 4. Demographics, Systemic Agents, and Systemic Disorders Affecting the Status of the Patient

Demographics		
Age	Occupation	Local environment
Gender	Physical activity	Geography
Habitus	Compliance	Nutritional access
Race	Self care	
Support systems	Socioeconomic status	
Systemic Agents That Affect Wound Repair		
Radiation therapy	Dialysis	Immunosuppressives
Transfusion	Immunomodulators	Corticosteroid
Cytotoxic agents	Cytostatic agents	Vasoactive drugs
Hormones	Anticoagulants	
Antimicrobials	Illicit drugs	
Nonsteroidal anti-inflammatory agents	Apheresis	
Concurrent Systemic Disorders		
Diabetes mellitus	Arteriosclerotic vascular disease	Neoplasia
Hypertension	Cardiac disease	Connective tissue disease
Venous disease	Trauma	Stress (local/systemic)
Cutaneous disease	Hypersensitivity	Musculoskeletal disease
Renal disease	Hepatic disease	Systemic infection
Gastrointestinal disease	Immunosuppression	Serum protein abnormalities
Endocrine disease	Neurosis/psychosis	
Septic shock	Pulmonary disease	

quantifiable measurements. The evaluation of the healing process is more difficult because it is a dynamic process: it requires ongoing, systematic, consistent evaluation. Ideally, this involves reassessment of wound extent, wound burden, and wound severity. The selection of parameters and the frequency of evaluation should be defined and appropriate to the process that is being observed. The modes of evaluating this process include assessment of changes in the following: angiogenesis, inflammation, fibroplasia and restoration of the connective tissue matrix, wound contraction, remodeling of the wound, epithelization, and differentiation (Tables 2 and 3). Synthesis of the collected information should be used to determine the progress of healing.

IMPLEMENTATION OF THESE GUIDELINES

Wounds Involving the Skin

The preceding definitions and guidelines were designed to be applicable to any wound. To illustrate the use of these guidelines, we will use wounds involving the skin as a paradigm. These wounds disrupt the epidermis and dermis and result in loss of barrier function. They may originate from forces internal or external to the skin.

To assess the extent, an accurate assessment of these wounds is based on observing structures and tissues involved. The assessment of extent includes the following: perimeter, maximum dimensions of length and width,

surface area, volume, amount of undermining, and determination of tissue viability (Table 1). Invasive methods may be necessary to quantify the extent of a wound. The tissue levels that may be involved in the wound are the epidermis, dermis, subcutaneous tissue, fascia, muscle/tendon, or bone/viscera.

Wounds involving the skin, like any wound, can be further described by various attributes. Assessment of skin wound attributes should begin with a thorough history and physical examination of the wound (Table 2). The examination includes careful description of the wound's appearance and can also include the following: wound location; description of the periwound skin and cutaneous appendages; wound color, both in a dependent and elevated wound position; capillary refill; venous filling; bruits and pulse status; varicosities; the presence of bleeding; erythema; edema; induration; fibrin; necrosis in the wound; surrounding gangrene; exudate; odor; lymphangitis; the presence of joint abnormalities; the historic origin of the wound; and a description of both spontaneous and induced pain. It is important that these parameters be quantified and recorded. There are a number of noninvasive and invasive technologies that can assist in quantifying attributes (Table 3).

Healing of Wounds of the Skin

The simplest method for evaluating healing in wounds involving the skin is to examine the wound and determine if it is minimally, acceptably, or ideally healed. An ideally healed wound of the skin results in a return to normal anatomic structure, function, and appearance that includes a fully differentiated and organized dermis and epidermis with intact barrier function. An acceptably healed wound is characterized by epithelization capable of sustaining functional integrity during activities of daily living. A minimally healed wound is characterized by the restoration of epithelial coverage that does not establish a sustained functional result and may recur.

Evaluation of wounds involving the skin requires ongoing, systematic, consistent assessment of wound burden and wound severity. This involves quantifying changes in extent (Table 1) and attributes both clinically (Table 2) and by using reproducible appropriate technologies (Table 3). These changes should always be correlated with changes in the status of the host.

SUMMARY

The escalating physiologic, psychological, social, and financial burden of wounds to patients, families, and society demands redress. The first step in the solution of this problem is agreement on definitions of wounds and wound healing, their assessment, and their evaluation. The definitions and guidelines described will enhance communication among all elements of society dealing with this problem. It is vital that the quality of clinical and technologic observations be as stringent as outlined in these guidelines. Once these uniform definitions and guidelines become standard, they can be used for determining standards of care, designing and implementing health care policy, addressing reimbursement issues, and setting end points for studies.

The broad applicability of these definitions and guidelines provides a framework for future consensus development regarding specific wound types involving hard and soft tissues, models, and technological assessment tools.

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During the preparation of the manuscript, Dr Pecoraro lost his valiant battle with cancer. His tireless efforts offered under the most adverse circumstances were an inspiration to us. We dedicate this article to his memory.

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CHAPTER 24

Wound Environment: Implications from Research Studies for Healing and Infection

Stephen C. Davis; Robert Perez, PhD; Fotios Andreopoulos, PhD

Objectives

The reader will be challenged to:

- Understand the importance of delivery systems for topical agents used in wound healing and infection
- Discuss the role of bacterial biofilms and the occurrence of antibiotic-resistant bacteria
- Present potential ways to disrupt and/or treat bacterial biofilms.

Introduction

Although ideal wound healing and infection studies should be performed using human subjects, it is not always practical or ethical. One of the difficulties lies in obtaining enough subjects with similar or identical situations to conduct well controlled studies. Additionally, studies on humans are impaired by the methods of analysis the researcher can perform. For example, it is impractical to biopsy a human subject at various timepoints throughout the healing process in order to properly conduct histological examination of the wounds. Ethical considerations prevent the intentional infection of a wound on a human or the use of an untreated control subject. Animal models have become a valuable commodity to the researcher because of these difficulties. Although small animal (eg, mouse, rabbit, guinea pig) models have been used, these animals have striking differences to humans. Small mammals tend to have dense fur with relatively thin epidermal and dermal layers. Additionally, they heal mostly by wound contraction instead of re-epithelization. Our animal model of choice is the pig, since pig skin is structurally similar to human skin, including similar epidermal thickness and dermal-epidermal thickness ratios. Additionally, pigs and humans share similar patterns of hair follicles and blood vessels. Biochemically, pigs contain dermal collagen and a dermal elastic content more similar to humans than other commonly used mammals.[1]

The purpose of this chapter is to discuss the importance of delivery systems for active agents (eg, growth factors) and review the possible role of bacterial biofilms and the occurrence of antibiotic-resistant bacteria. We will also present some preclinical data using our porcine wound healing and infection models, which may lead to new therapeutic agents for the clinic.

Growth Factor Delivery Vehicles in Wound Healing

Growth factors play an important role in tissue repair by participating in the regulation of cell proliferation, migration, differentiation, and organ development. Exogenous administration of growth factors has been identified as a potential therapeutic approach in accelerating the rate of acute and chronic wound healing. Nonetheless, their susceptibility to enzymatic degradation, short half-lives, and the need to maintain adequate pharmacological levels at the injured site have limited the widespread usage of bolus peptide delivery in cutaneous repair.[2-7] Improvements on existing controlled delivery technologies or newly

Davis SC, Perez R, Andreopoulos F. Wound environment: implications from research studies for healing and infection. In: Krasner DL, Rodeheaver GT, Sibbald RG, eds. *Chronic Wound Care: A Clinical Source Book for Healthcare Professionals*. 4th ed. Malvern, Pa: HMP Communications, 2007:205–213.

designed controlled delivery vehicles that protect the peptides from deactivation, prolong their contact time, and mimic the endogenous growth factor profiles are essential for the successful employment of exogenous growth factors in wound care. In recent years, several strategies and materials have been considered for the effective delivery of growth factors to the wound site, but the appropriate mode for making these peptides available at the desired site still remains unclear due to variability of wound size and severity and the underlying cause of wound pathology.[8–13]

Given the complex nature of the wound healing process, an effective delivery vehicle for cutaneous regeneration must address several key issues. First, the biomaterial used to construct the vehicle must be compatible with the skin (ie, mimic the surface of the skin), must be nontoxic, and must provide an adequate layer of protection from infiltrating bacteria. Second, the vehicle should be designed to release the growth factor at controlled rates that allow its appropriate spatial and temporal exposure to the injured or diseased tissue. Third, the vehicle should stabilize the embedded/encapsulated growth factor for the duration of the treatment and release the peptide in an active form. The biomaterials selected for the development of the peptide vehicle vary widely depending on the application and the nature of the growth factor being used. Synthetic polymers, such as polyethylene glycol, polyvinyl alcohol, polylactic acid, or polyglycolic acid and its copolymers, or natural polymers, such as proteins (eg, gelatin, collagen), sugars (eg, cellulose), and glycosaminoglycans (eg, heparin, hyaluronic acid), are commonly used for the construction of growth factor delivery vehicles. Vehicles can also be designed to undergo nonspecific (eg, hydrolysis) or tissue-responsive (eg, pH, enzyme concentration) biodegradation. The rate of biodegradation can be controlled in a variety of ways to occur over a period of days to months, and it can be synchronized to disappear completely once all of the encapsulating peptide has been delivered and new tissue is formed. Factors affecting this rate include the nature of the biomaterial (synthetic versus natural), the degree of crystallinity and molecular weight of the polymer, the crosslinking density, and the shape and porosity of the carrier construct.

Some of the most promising growth factors currently investigated in wound healing applications include basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF). Epidermal and basic fibroblast growth factors have received significant attention for their potential to expedite the healing process. The efficacy of both factors necessitates continuous exposure to the wound cells and depends upon the dosage and mode of delivery.[14–16] Epidermal growth factor is a small polypeptide of 53 amino acids with a molecular weight of 6,000 Da, and it shares 42% sequence homology with the functionally related transforming alpha (TGF-α). Epidermal growth factor is f all bodily fluids and is widely distributed in t nal tract and the skin. It is mitogenic for mo sues, fibroblasts, and endothelial cells. Epiderm tor accelerates epidermal healing of partial- wounds in pigs and donor sites of partial- grafts in humans.[14,17,18] In addition, it has be stimulate corneal regeneration in animals an to increase tensile strength in incisional wou data collected from clinical trials demonstra effectiveness of EGF necessitated multiple d (a liquid vehicle, such as saline, was used), need of vehicles that could stabilize the peptic spatial localization.[7,19] The importance of th vehicle in exogenous growth factor thera demonstrated in several studies. Ulubayram e that the controlled delivery of EGF from gela enhanced the healing of full-thickness skin wo parison to bolus peptide administration. Alter longed release of EGF was achieved by conj with a polyethylene glycol chain and then enc pegylated growth factor within polylactic-co- microspheres. The polyethylene glycol (PEG) enhanced the EGF stability within the poly(D glycolide) (PLG) microspheres and reduced th effect.[7] To ensure the prolonged contact of E wound site necessary for signaling, Kuhl and Gr designed a controlled delivery system by tether flexible water-soluble polymeric spacers onto strate. The growth factor retained its activity and elicit both mitogenic and morphological cha tested cell cultures.

Basic fibroblast growth factor is a single ch (16,000 Da) composed of 146 amino acids an proliferation, differentiation, and numerous ot functions in cells derived from the mesoderm a toderm.[20–26] It is a potent angiogenic factor in *vitro* and is mitogenic and chemotactic for fib endothelial cells.[26] Administration of exogenou been proven beneficial for the healing of acute ic wounds in animals and clinical trials. Howe EGF, major limitations that prevent the clinical of bFGF for the treatment of acute and chron include its susceptibility to enzymatic and therm tion, its short retention time at wound sites, and an ideal method of administration.[17,26] A numb trolled delivery vehicles have been developed to the shortcomings of bFGF therapy in a clini Slavin et al[27] reported that topical application encapsulated into red blood cell ghosts exerted effect on incisional wound strength in rats. Inter

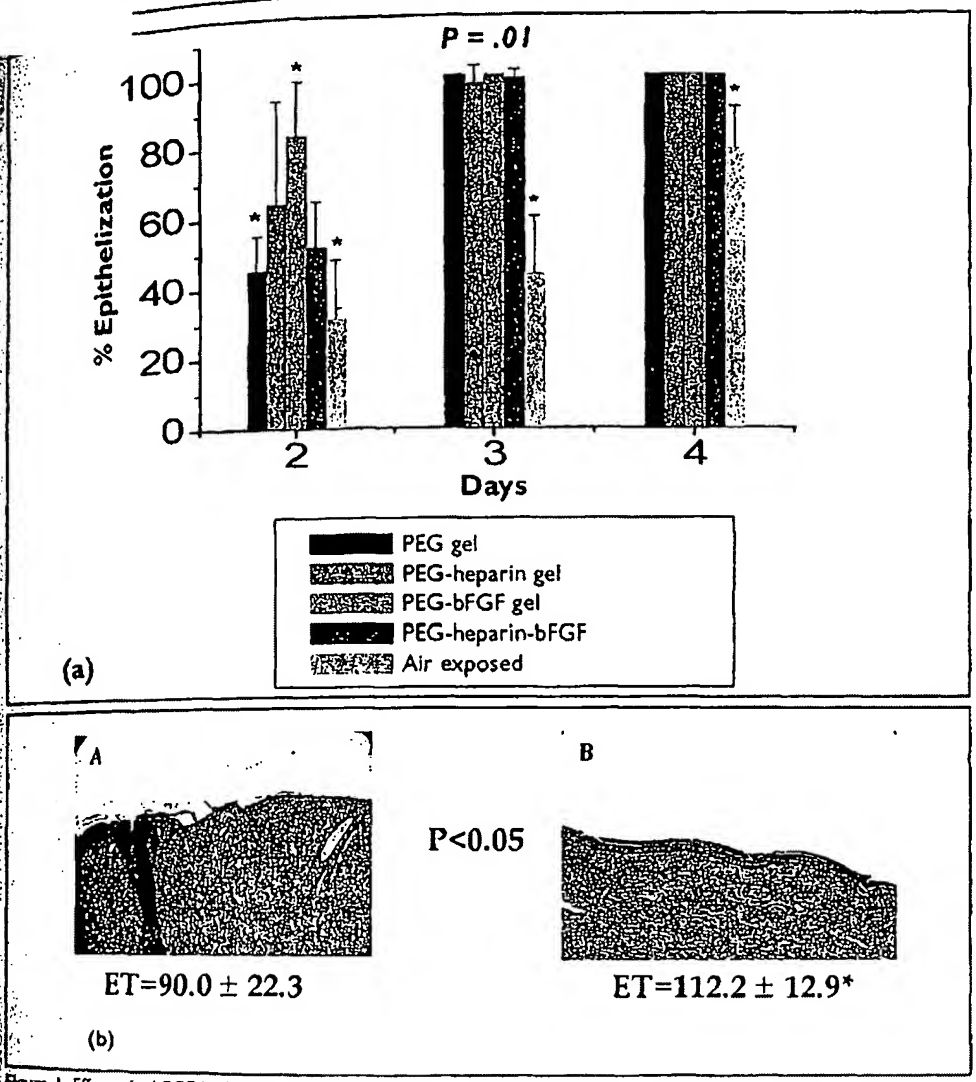

Figure 1. Effect of a bFGF hydrogel on partial-thickness wounds. (a) Deep partial-thickness wounds (10 mm x 7 mm x 0.6 mm deep) were made on the back of pathogen-free female pigs. The treatment groups included polyethylene glycol-heparin hydrogel scaffolds releasing bFGF. The concentration of the precursor solutions (volume of 100 µl) was [PEG-nitrocinnamate] = 0.18 g/mL, [heparin-nitrocinnamate] = 20 µg/ml, and [bFGF]= 5 g/ml. The solution was placed on the top of the wounds and was crosslinked to a gel film under 90 seconds exposure to 365 nm long wave UV irradiation. (b) H&E stains of skin biopsies to assess the epithelial thickness following treatment of deep partial-thickness wounds with bFGF-releasing PEG-based hydrogels. Panel A depicts the epithelial thickness (ET) of the completely epithelized wounds after 4 days of treatment with bFGF-releasing PEG hydrogels while panel B depicts the epithelial thickness of healed wounds that were treated with bFGF-releasing PEG-heparin hydrogels. The results indicate that the presence of heparin might potentiate the activity of bFGF leading to an increased epithelial thickness.

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Acceleration in healing was only observed when the red blood cell ghosts were used as a vehicle for the slow release of bFGF to the wound site. The unique ability of heparin to bind to bFGF and other basic polypeptides has been used to develop drug delivery systems for the controlled release of heparin-binding growth factors.[12,28] For example, Gospodarowicz and Cheng[29] showed that acidic fibroblast growth factor (aFGF) and bFGF coupled with heparin

were protected from heat and acid deactivation, while Venturi et al[9] reported the stabilization of bFGF in the presence of heparin at high temperatures. Edelman et al[9] demonstrated that the binding of bFGF to heparin-Sepharose beads allowed prolonged storage in a microspherical controlled releasing matrix, while Sakiyama-Elbert and Hubbell[10] reported that fibrin-heparin matrices can enhance nerve regeneration by controlling the release of bFGF at the injury site. They also indicated that the bioactivity of bFGF depends upon its release kinetics. DeBlois et al[11] also have utilized the heparin-bFGF binding affinity to design collagen-based bioactive materials for connective tissue replacement. The rapid clearance rate of the bFGF from the injured site also could be augmented by its complexation via electrostatic interactions with other polyionic molecules like gelatin.[9] Similarly, work performed in our laboratory corroborates the significance of the vehicle's properties and the potentiating effect of heparin on bFGF activity and its release kinetics.[4] Photoresponsive PEG-based hydrogels were prepared from the crosslinking of PEG-nitrocinnamate polymers upon short exposure to long wavelength UV irradiation (365 nm). The rate of release and activity of the encapsulated bFGF was a function of scaffold properties (eg, crosslinking density), heparin content, and growth factor loading concentration. Preliminary *in-vivo* porcine studies demonstrated the feasibility of the bFGF-releasing PEG hydrogels as favorable wound healing dressings (Figure 1a) and the effectiveness of heparin in stabilizing bFGF (Figure 1b).

Exogenous PDGF delivery for the treatment of acute and chronic wounds has gained much attention as an alternative wound healing therapy. Platelet-derived growth factor (~ 30,000 Da) is a dimeric protein composed of A and B chains, each one of which is made up of more than 100 amino acids. Platelet-derived growth factor is released from platelets, macrophages, endothelial cells, and fibroblasts, which are all involved in wound healing. It is among the first cytokines to reach the wound site as it is released from the alpha granules in the platelets, acting as a strong mitogen for fibroblasts and potent chemoattractant for macrophages and neutrophils. It is the only commercially available, topically applied growth factor that is approved by the US Food and Drug Administration (FDA) for the treatment of diabetic foot ulcers. Interestingly, the polypeptide product, Regranex (Johnson & Johnson Wound Management, Somerville, NJ), utilizes a gel formulation (0.01% recombinant PDGF-BB in a carboxymethylcellulose gel) for the delivery and stabilization of PDGF.[35] Regranex therapy has also shown promise for the treatment of deep pressure ulcers in 2 separate clinical, randomized, double-blind trials.[36,37]

Since single growth factor therapy has not been uniformly successful for the treatment of c
wounds, combination or sequential cytok
been proposed. Lynch et al[38] demonstrated
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hyaluronic acid (HA) had a synergistic effect
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HA films in a mouse model. In a separate st
al[44] reported that the co-delivery of keratino
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tion of PDGF-BB and bFGF in rat and rabb
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rico release during natural tissue regeneration.

Bacterial Biofilms

In nature, free-floating (or planktonic) bacteria
to and colonize surfaces and form bacterial biofil
are complex structures consisting of microbia
cells embedded in a self-produced extracellular
hydrated extrapolymeric substances (EPS), whic
versibly attached to biological or nonbiological s

Our laboratory has studied the effects of vario
crobial agents on both types of bacterial phenotyp

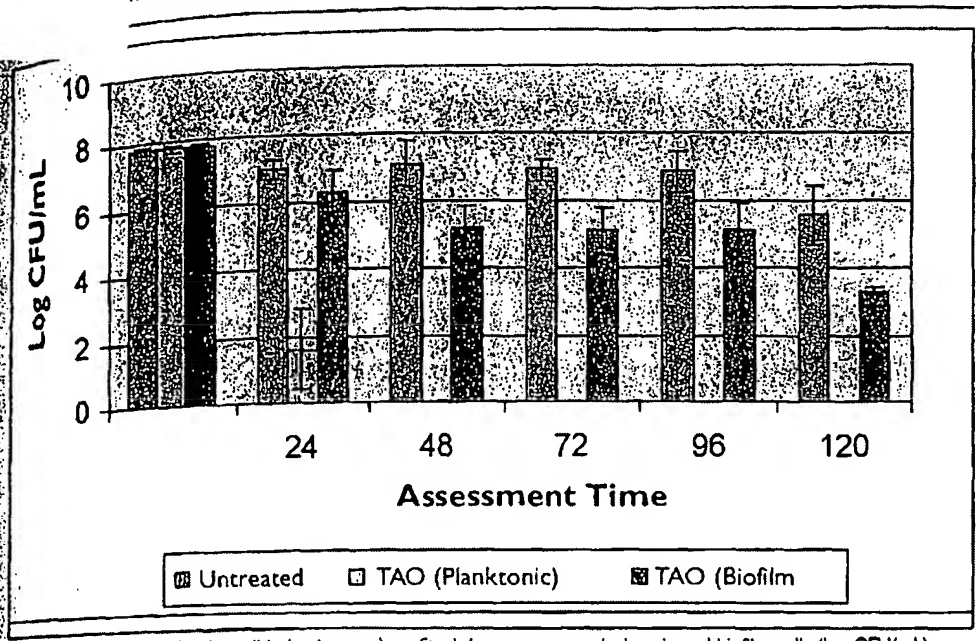

Figure 2. Effect of TAO (triple antibiotic ointment) on *Staphylococcus aureus* planktonic and biofilm cells (log CFU/mL).

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...ating (or planktonic) bacteria ... ...faces and form bacterial biofilm... ...tures consisting of microbial ... ...a self-produced extracellular ... ...meric substances (EPS), which ... ...biological or nonbiological ... ...as studied the effects of vario... ...oth types of bacterial phenoty...

...omic versus biofilm) using a porcine model. In the porcine wound model, planktonic bacteria can be studied by inoculation of wounds with a known amount of pathogen followed by immediate treatment (within a 20-minute period, while the bacteria are still "free floating") with antimicrobial test agents. To study the effect of agents on biofilm bacteria, we allow the bacteria to colonize for a period of 24–72 hours. These wounds are covered for the duration with a polyurethane film to allow the bacteria to firmly attach and establish a biofilm. After formation of the biofilm, the polyurethane film is removed, and treatments are applied directly to the wound. Using both of these models (planktonic and biofilm), we found that an over-the-counter triple antibiotic ointment showed significant reduction in planktonic bacteria with complete eradication by 48 hours (Figure 2). In contrast, it took several days to see a modest log reduction in bacterial counts, and complete eradication of the biofilm bacteria was never achieved. Results from this study suggest that continuous treatment is needed to reduce and, possibly, eliminate bacteria in wounds with established biofilms.

Biofilm Resistance

A reason for antimicrobial resistance within biofilms appears to be due to close cell-cell contact that permits bacteria to more effectively transfer plasmids to one another. These plasmids can encode for resistance to several different antimicrobial agents.[9] Another factor contributing to resistance is quorum sensing, which can aid in signaling bacteria to adopt a slow-growing state when placed in an environment with adverse growth conditions. When in this state of intermission, bacteria are less susceptible to antimicrobial attack.[12] The biofilm also provides physical protection to bacteria, since most antimicrobial agents are ineffective at penetrating the biofilm. This serves to decrease the concentration of agents reaching the bacterial cells within the biofilm and, as a consequence, the efficacy of the agents.[13]

Biofilms also appear to have an antiphagocytic property, which renders white blood cells present within the matrix ineffective.[9] There appears to be an additional component within the polysaccharide that inactivates and traps complement and host antibodies. These factors lead to an accumulation of host immune factors, resulting in host tissue damage and chronic inflammation.[12]

Potential Ways to Remove Bacterial Biofilms from Wounds

There are 3 main ways to possibly prevent, reduce, or treat biofilms. These include 1) preventing the bacterial attachment, 2) preventing biofilm formation, 3) disrupting the biofilm to allow penetration of topical antimicrobial agents, 4) interfering with quorum sensing, and 5) enhancing dispersion of bacteria from biofilms so the planktonic bacteria can be more easily destroyed.

The study of preventing bacterial attachment has been around for a long time, especially in dentistry. The attachment and colonization of subgingival sites by bacteria is a critical step in the initiation of periodontal diseases.

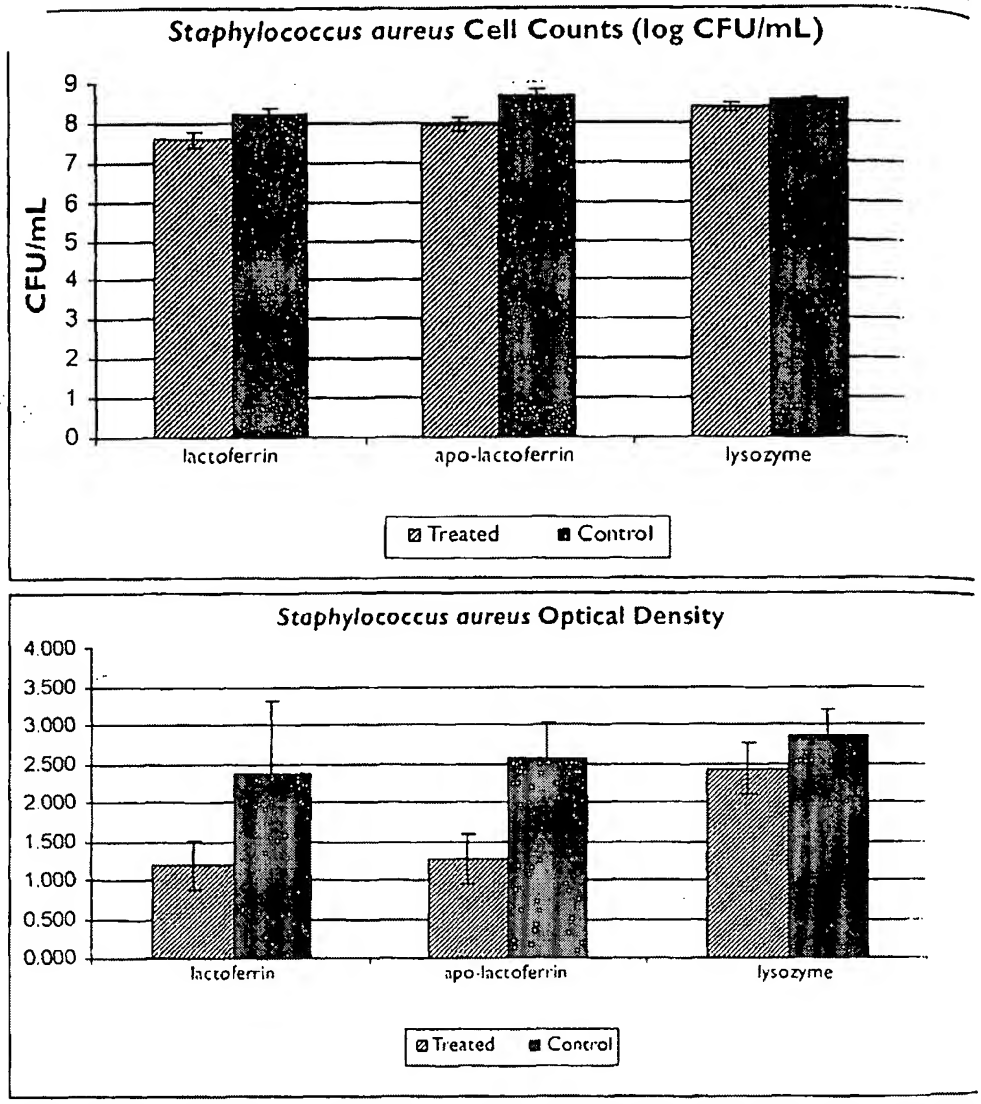

Figure 3. Effect of lactoferrin, apo-lactoferrin, and lysozyme on *Staphylococcus aureus* biofilms (cell counts and opt density).

However, various compounds have been shown to significantly prevent attachment of bacteria to the surface of teeth.[9] In orthopedic surgery, the treatment of implanted devices with antimicrobial compounds has successfully reduced bacterial adhesion.[3] It is plausible to imagine reduction of bacterial colonization by topical agents, which are applied to a wound after wound bed preparation. However, the effect of these agents on the wound healing process would also need to be examined.

We have recently studied the effect of lactoferrin, a glycoprotein that belongs to the iron transporter family.[1] protein is found in milk and exocrine secretions and released by neutrophils during degranulation. Addition it has been shown to have antimicrobial and anti-inflam tory properties.[2] Recently, lactoferrin has been investig ed as a possible treatment for biofilm formation. Early st ies have demonstrated its utility in preventing *Pseudom aeruginosa*, *Staphylococcus aureus*, and *Staphylococcus epider* from forming biofilms. However, the protein's effect established biofilms is less impressive.[3] The proposed me

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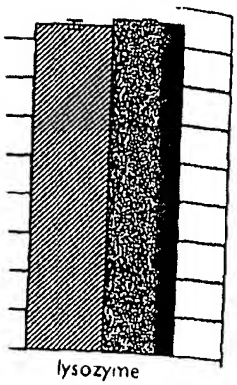

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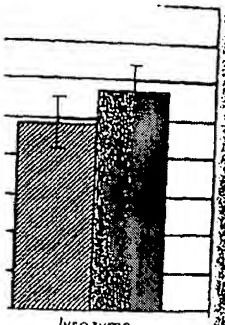

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by which lactoferrin inhibits biofilm formation involves chelating iron, which in turn causes a low iron level, triggering bacteria to "twitch," thereby inhibiting bacterial adhesion.[38] Recently, our group examined the effect of lactoferrin, apo-lactoferrin (the iron-depleted form of lactoferrin), and lysozyme (an enzyme that can destroy bacterial cell walls and is found in the mucosal membranes) on *Staphylococcus aureus* biofilm mass. The lactoferrin we used was a recombinant human form supplied by Ventria Biosciences (Sacramento, Calif).[39] Using optical density as a measure of biomass, we found that both recombinant human lactoferrin and apo-lactoferrin reduced biomass material (*in vitro*) by 45.5% and 49.1%, respectively (Figure 1). Lysozyme-treated bacteria showed insignificantly lower biofilm formation than the control. Both lactoferrin and apo-lactoferrin showed small but significant decreases in viable biofilm-associated cell counts as well. This indicates that these substances appear to have an inhibitory effect, in addition to bactericidal effects, on biofilm production of *Staphylococcus aureus*. However, it is unclear if the reduction in biofilm mass was due to the decrease in cells present or a direct effect on the biofilm. From these results, one might conclude that lactoferrin would be effective for the treatment of chronic wounds. However, controlled clinical trials are still ongoing.

The idea of disrupting an established biofilm is attractive. This could be accomplished in a number of ways, including physical methods and/or the application of topical substances. Potential physical methods include debridement, electrical stimulation, and ultrasonic disruption. In addition to removal of the bacteria and the biofilm, debridement aids in the removal of necrotic tissue on which bacteria thrive. Electrical stimulation has been used throughout the years to assist penetration of various topical agents. For example, electroporation and electrophoresis have been shown to enhance the penetration of a photosensitizer.[39] Therefore, it is conceivable that electrical currents could aid in the delivery of topical antimicrobial agents through the physical barrier provided by the biofilm. The use of enzymatic agents provides an additional method for the penetration and disruption of biofilms. Enzymes can be used in combination with topical antimicrobials to help facilitate the antimicrobial action on the bacteria within the biofilm.

Another way of preventing or possibly reducing biofilm formation is interfering with bacterial communication (quorum sensing). For example, *Pseudomonas aeruginosa* imparts disease through many virulence factors, which can be activated or deactivated by various quorum-sensing based systems, such as *Pseudomonas* quinolone signal (PQS). Studying PQS's ability to alter gene regulation and virulence in *P aeruginosa* may result in promising target therapeutic agents aimed at decreasing the pathogenesis of this bacterium.[40]

Finally, factors that control bacterial dispersion are now being closely studied. Cells that become dispersed and, therefore, are not living in a biofilm state are easier to eradicate. A recent study has shown that dispersal of *Pseudomonas aeruginosa* (PAO1) from biofilms is induced by a sudden increase in carbon substrate availability. Additional knowledge of signal transduction in biofilm dispersion by screening of differential gene expression and phosphorylated proteins associated with dispersion may offer new targets for the treatment of biofilm-related infections. This could ultimately guide us to the development of novel approaches for biofilm control with clinical applications.[41]

Conclusion

The development of sophisticated delivery vehicles is critical in augmenting the effectiveness of exogenous growth factor therapy in tissue repair. Strategies to deliver growth factors need to consider the complex nature of the damaged or injured tissue and be responsive to the different stages of the healing process. One of the limitations of current growth factor delivery vehicles is that they all focus on single peptide delivery. However, it is clear that combinations of growth factors or the sequential delivery of multiple growth factors that mimic the endogenous growth factor release profiles during normal healing may well be the future answer to accelerating the healing process. Along with identifying the key biological signals triggering the different stages of normal or chronic wound healing, new technologies that are focused on delivering multiple growth factors via polymeric vehicles, gene expression, or cell transplantation have become a critical area of research that will play a significant role on the success of exogenous growth factor therapy in tissue regeneration. In addition, the use of well controlled animal models to study the effectiveness of these delivery systems is of utmost importance.

Biofilm formation in wounds may be responsible for the duration and infectious complications of various types of wounds. The presence of biofilms also represents a major obstacle in effective treatment. Many studies investigating treatments for biofilm formation in chronic wounds are ongoing, but more are needed. While *in-vitro* study of novel approaches to control or eradicate biofilm formation are being performed, *in-vivo* testing is necessary since various factors (eg, wound fluid, proteases, growth factors) need to be taken into consideration to determine the true efficacy of these agents.[42]

Acknowledgments

We wish to acknowledge the support of the National Institutes of Health (NIAMS: RO3-AR50518) and Ventria Biosciences.

Exhibit B

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Take Home Message for Practice

- Good delivery systems are essential for effective active agents and a variety of substances are available.
- It is important to understand how biofilms may be involved in order to prevent or eradicate wound infections.

Self-Assessment Questions

1. Vehicles are used for the following reasons:
   A. Protect active agents from becoming deactivated
   B. Control the delivery rate of the active agent
   C. Make the active agent less active
   D. A and B only

2. Biofilm formation involves all of the following EXCEPT:
   A. Attachment to a surface
   B. Production of a self-produced extracellular matrix
   C. Only planktonic bacteria
   D. Quorum sensing

3. Optimal vehicles should:
   A. Be compatible with the skin
   B. Be nontoxic
   C. Be designed to release the active agent at a controlled rate
   D. Stabilize the embedded/encapsulated active agent
   E. All of the above

4. Quorum sensing is:
   A. How the bacteria attach to each other
   B. How bacteria communicate to multiply or not
   C. How bacteria are killed by macrophages
   D. Has nothing to do with bacteria

5. Planktonic bacteria refers to:
   A. Bacteria that need food
   B. Bacteria that are in large colonies
   C. Bacteria that are free-living or floating
   D. Bacteria that are ready to die

6. All but 1 of the following factors may contribute to bacterial biofilms' antibiotic resistance:
   A. Quorum sensing
   B. Genetic alternations in bacteria
   C. The self-produced extracellular matrix acts as a physical barrier
   D. Electrical fields created by the bacteria

Answers: 1-D, 2-C, 3-E, 4-B, 5-C, 6-D

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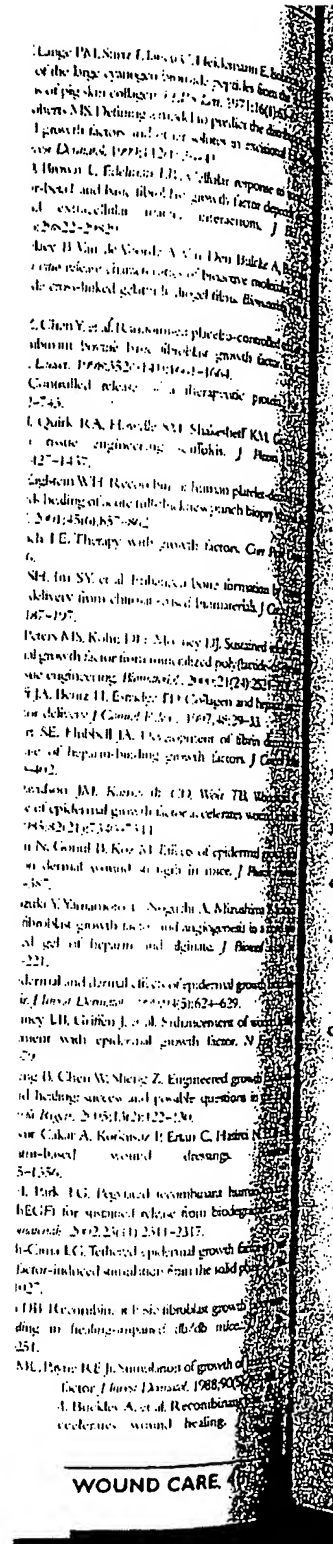

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CHAPTER 28

Dressing Decisions

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S2 Moisture Hydration Absorption

Objectives

The reader will be challenged to:

- Integrate decision-making strategies for topical dressings into clinical practice
- Categorize dressings by topical wound care goals
- Compare topical wound management strategies based on the topical wound care objective.

Introduction

Arguably, dressing decision choices present the clinician with one of the greatest and most confusing challenges in wound care. It is estimated that there are more that 2,400 topical dressings available to assist in healing a wound. Some clinicians would argue that the choice of the topical dressing is one of the least important aspects of providing good wound care. Others would assert that the most important aspect of wound care, especially chronic wound care, is the identification of wound healing failure. If the clinician can correctly identify why a wound is failing to heal, the appropriate strategies to correct this can be developed. For example, wounds that are ischemic must have an improvement in circulation and oxygenation in order to heal. This can be accomplished through revascularization procedures and perhaps hyperbaric oxygenation. The plantar surface diabetic foot wound can have the best topical care, yet if it is not offloaded, the repetitive trauma of walking will likely lead to failure to heal. Wound healing is a complex process, involving strategies that address the underlying etiology (or etiologies) as well as strategies that optimize the host and wound environments for healing. Dressings are one of several interventions that will be required for each individual with a wound. Dressings do not heal wounds; properly selected dressings enhance the body's ability to heal the wound.

The ideal dressing keeps the wound bed moist while keeping the periwound tissue dry. It removes excess exudate but does not desiccate the wound. It provides a barrier against bacteria and particulate matter and allows for gaseous exchange. Creation of the optimal wound environment is a benefit of appropriate dressing selection. Appropriate dressings increase healing rates. They reduce pain and decrease infection rates. The appropriate dressing is also cost effective and affordable. For further information on dressing performance parameters, see Chapter 27.

Inappropriate dressing selection allows the periwound to macerate. In addition, the wrong dressing can lead to tape tears due to inappropriate methods to secure the dressing in place. Inappropriate dressings often increase pain. An inappropriate dressing can also lead to delayed wound healing related to wound bed injury, hypergranulation, or dehydration.

Many strategies have been developed to assist the clinician in making decisions as to what dressing to apply to what wound. Clever systems that make recommendations based on the color of the wound bed have been developed. All of these various decision trees help the clinician

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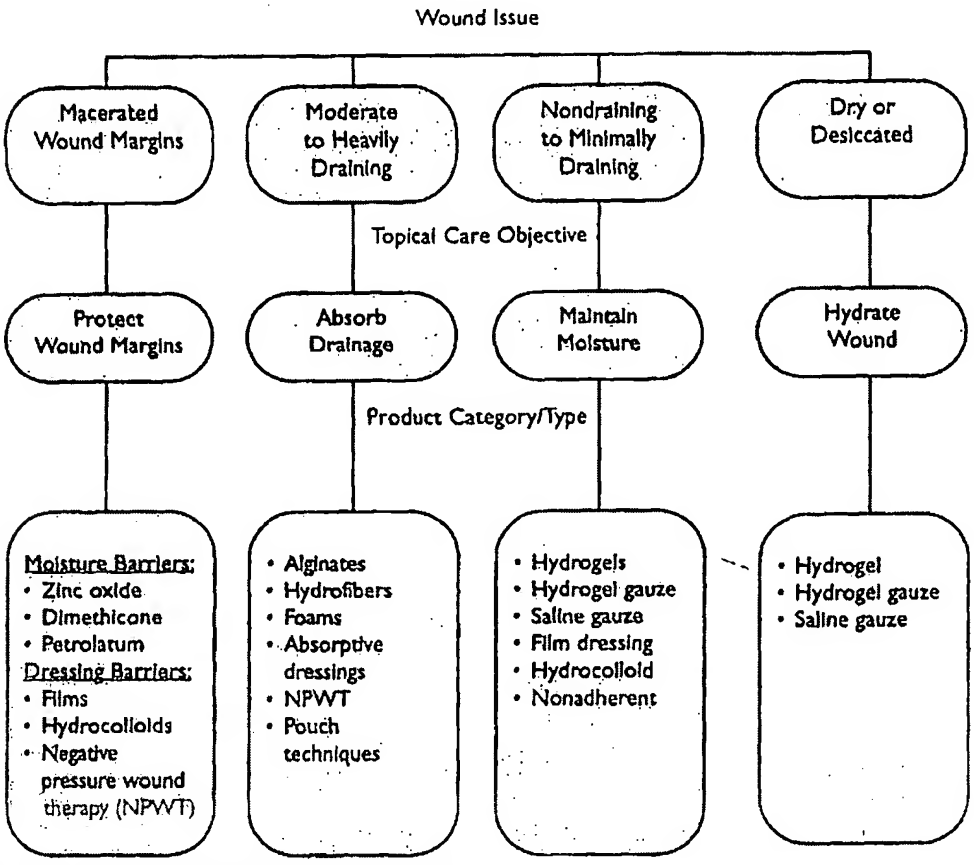

Figure 1. Optimizing the moist wound healing environment.

navigate the maze of topical wound care dressings that are now available. Each of these decision trees has merit and is worthy of study by the clinician.

Simply referred to as The Dressing Decision Tree, a decision tree developed by the author takes into account a variety of goals that the clinician may wish to accomplish through the selection of topical dressings. The 5 goals of topical care include: 1) optimization of the moist wound environment; 2) wound manipulation and stabilization, pressure reduction, and edema management; 3) activation of the wound environment; 4) control of infection and bioburden; and 5) quality-of-life improvement.

Optimizing the Moist Wound Healing Environment

One of the most important tenets of wound care is optimizing the moist wound environment (Figure 1). The original idea of moist wound healing gained prominence with the work of Dr. George Winter's publication in 1963 on the effects of air drying and dressings on the wound surface.[1] While the idea was not new, it was perhaps the seminal publication that explored the benefits of moist wound healing. Optimization of the moist wound environment considers these tenets and is the primary objective in topical dressing decisions.

The category of dressings included in this section comprises passive dressings that address wound issues of too much, not enough, or the appropriate amount of moisture in the local wound environment. These dressings provide topical wound care but do not cause a specific action to occur on a cellular level in the wound. These dressings serve 4 functions, including absorbing wound drainage, protecting the surrounding tissue from excess moisture, maintaining a moist wound, or contributing moisture to a wound.

Frequently, chronic wounds are first seen in a chronic inflammatory phase. This may result in the wound being

exudative. As such, the goal of dressing selection is two-fold: to absorb drainage and to protect wound margins from maceration. An adequate dressing that absorbs excess exudate would help to protect the wound margin; however, wounds that are highly exudative may require additional protection of the periwound area. Most commonly, the use of moisture barriers will assist in accomplishing this goal. A variety of moisture barriers exists, and selection is often determined by availability. Perhaps the most common barrier is zinc oxide. Other moisture barriers include dimethicone and petrolatum. Dressings may also provide protection of the periwound area. Film and hydrocolloid dressings provide structural protection from both moisture and adhesives. A final dressing strategy that protects the wound margin is the use of negative pressure wound therapy (NPWT).

The second goal in the management of exudative wounds is the absorption of drainage. Several dressing options will assist in meeting this goal. The most common dressings for exudate control are bulk gauze dressings. While these will perform well, disadvantages to using bulk gauze exist. The primary disadvantage is that once maximum absorption is reached, the gauze acts as a wet dressing and actually may increase the risk of maceration. Consequently, dressing change regimens typically require the dressing to be changed 2 or 3 times per day. In addition, there is concern that gauze may produce lint that remains in the wound, causing an inappropriate inflammatory response to foreign debris, or that aerosolizes, causing the dispersal of microorganisms in the local environment.

Alginate and hydrofiber dressings are excellent exudate absorbers. It is estimated that alginate dressings may absorb 40 times their weight in wound fluid. Alginate dressings are older dressings originally manufactured from seaweed. Due to the calcium content of these dressings, they also make good hemostatic dressings following debridement. One hydrofiber dressing is available. This dressing is used in the same manner as the calcium alginate dressing. Once in contact with wound fluid, the hydrofiber dressing converts to a gel, ensuring a moist wound environment. Removal of these products is easily achieved through wound irrigation. Both alginate dressing and the hydrofiber dressing require a secondary dressing. Appropriate secondary dressings may include gauze or sponge dressings depending on the amount of drainage. Daily dressing changes are usually required for alginate and hydrofiber dressings.

Another class of dressing that can be used to control exudate is the foam dressing. Numerous foam dressings are available to the clinician. The more desirable foam dressings are those that wick fluid unidirectionally away from the wound and do not wick moisture into the foam except for the area where the wound is draining. Foam dressings are versatile. In addition to absorbing large amounts of drainage, foam dressings also provide insulation to the wound. A secondary dressing to keep the foam in place is generally required. Newer foam dressings are lined with silicone to prevent trauma at wound dressing change.

Another strategy to control excess exudate is through NPWT. Negative pressure wound therapy works by applying a vacuum to the wound and thereby removing and containing excess moisture. Pouching is an older technique that allows excess wound exudate to freely drain into a pouch. Gauze added to the pouch may help contain the fluid, preventing it from leaving the pouch.

The third use of pasive dressings is for the maintenance of an optimally moist wound environment. These wounds may have minimal or small amounts of drainage. For those wounds with some drainage, choosing a dressing with some absorptive capability may be necessary but not to the degree that would occur with the dressings used in highly exudative wounds. The use of a highly absorbent dressing may actually desiccate a wound. If there is no exudate, a film dressing will maintain the moist environment. An advantage of using a film dressing is that the wound can be easily seen because the dressings are transparent. Films are gas permeable and allow for the evaporation of some moisture. These dressings are also good moisture barriers and function as secondary dressings to secure other dressings while preventing moisture from coming in contact with the primary dressing. Film dressings are also excellent in repelling environmental debris and dirt. Film dressings tend to be inexpensive. A disadvantage of using a film dressing is that it may be difficult to apply. To address this, manufacturers have created novel application approaches, such as windowing the film or providing a secondary firm outer layer that can be removed once the dressing is in place. Film dressings can be changed on an as-needed basis. If small amounts of exudate pool under the dressing, a sterile needle can be used to carefully puncture and express the exudate from under the dressing. The puncture site can be patched with a small piece of film, allowing removal of exudate without disturbing the wound bed. Film dressings should be changed at least weekly.

Hydrocolloid dressings are also dressings that will maintain a moist wound environment. These dressings were originally formulated from plant pectin. The original hydrocolloid dressings tended to deteriorate over time and had a foul odor. New technology creates hydrocolloid dressings from synthetic materials, thus the problem of dressing deterioration is reduced; however, since the hydrocolloid dressing is generally an occlusive dressing, odor sometimes remains a problem. Hydrocolloids form a gel at the wound-dressing interface and provide a minimal amount of absorption. They protect the wound from environmental contaminates, including moisture, and reduce the risk of infection. Hydrocolloid dressings provide superior autolytic debridement; however, as they tend to be

occlusive, care should be taken in using them in diabetic ulcers. Many clinicians will avoid their use in a diabetic wound of the lower extremity because of the potential risk of anaerobic infection. Another concern with hydrocolloid dressings is difficulty in application if the dressing is cold or even at room temperature. Placing the packaged dressing in a pocket or under the patient will warm the dressing and ease application. Hydrocolloids, once applied, tend to adhere aggressively, so care should be taken during removal to prevent injury to the periwound skin. One method of removal is to moisten the adherent edge with a normal saline-moistened gauze as it is being removed. Hydrocolloid dressing change regimens are typically 3 times per week.

The nonadherent class of dressings will also maintain the moist wound environment. Most of these dressings have no absorption potential, with the exception of those dressings with a nonadherent contact layer comprised of woven polyamide netting with a cotton filling. A similar low adherent dressing material made from ethylene terephthalate is perforated to allow for the passage of small amounts of exudate. A secondary dressing, such as gauze, is necessary with this dressing. These types of nonadherent dressings are changed daily or as dictated by exudate. Should they adhere to the wound base, moistening with saline will facilitate removal. Other nonadherent dressings have no absorption capability and should not be used in wounds that have exudation, unless a secondary dressing is used to absorb and prevent maceration. Other nonadherent dressings include petrolatum and silicone dressings. Petrolatum and silicone dressings are useful in those wounds where contact with a dressing may damage the wound surface. Handling these products can prove challenging as they are tacky. Moistening gloves with water helps keep the tendency to stick under control. Topical medications, such as steroids and antimicrobial agents, can be applied under a petrolatum or silicone dressing. Topical medications can be applied on top of the silicone dressing. Both of these dressings require a secondary dressing. Silicone dressings are particularly useful in skin tears. Dressing regimens are designed for secondary dressing changes, not petrolatum or silicone dressing changes. Petrolatum gauze dressings are usually changed 3 times per week. Silicone dressings are usually changed weekly.

The same dressings are used for wounds with no drainage or those that are dry or desiccated. While the goal in nondraining wounds is to maintain the moisture content of the wound, the goal for the dry or desiccated wound is to hydrate the wound. The mainstay of this activity is the use of hydrogel. Hydrogel dressings come in 2 forms. Sheet hydrogel dressings are formed hydrogel dressings with low adherence to the wound surface. These dressings combine amorphous hydrogel with an agar. These dressings are particularly useful in painful wounds because they cool the wound. They offer a limited amount of exudate absorption. Hydrogel sheets are biodegradable and will deteriorate in the wound if left in place for prolonged periods of time. Sheet hydrogel dressings are changed 3 times per week. Amorphous hydrogels are in a viscous form and are relatively common. Many of these products have other components including collagen, plant extracts, and trace elements; however, the primary use of amorphous hydrogel is to hydrate a dry or desiccated wound. Like hydrogel sheets, amorphous hydrogel dressings are soothing and will help alleviate pain. They are also an excellent choice for autolytic debridement. A secondary dressing is always required. To facilitate application of the hydrogel, applying the hydrogel to the secondary dressing then applying that dressing to the wound will ease the application. The use of applicators or tongue depressors also facilitates application directly to the wound surface. Amorphous hydrogel should never be used as a wound filler by itself—it should be used to layer the wound surface. If it is necessary to fill the wound, the use of hydrogel gauze would be appropriate. Removal of amorphous hydrogel is easily accomplished by irrigating the wound. Amorphous hydrogels are usually changed daily.

Another method of hydrating a dry wound is the use of saline gauze. These are not wet-to-dry dressings. A wet-to-dry dressing in chronic wound care is a debridement technique. Saline gauze should be wet-to-moist. Inherent problems with saline gauze are that these dressings must be changed 2 to 3 times a day. The impact this has on caregiver time and the inconvenience to the patient make saline gauze dressings impractical. In addition, an open bottle of saline is also known to culture bacteria within 24 hours. To avoid wound contamination, saline should be discarded daily. As a result, saline dressings can become expensive.

Wound Stabilization, Pressure Reduction, and Edema Management

The second objective in topical dressing decision involves wound stabilization, pressure reduction, and edema management (Figure 2). These dressings can be referred to as mechanical dressings because they alter the physical aspect of the wound.

The first issue addressed by mechanical dressings is an issue frequently seen in wounds requiring passive dressings, ie, cavernous, undermined, and tunneled wounds. The goal of topical care is to fill or obliterate the dead space to prevent premature closure of a wound and subsequent abscess formation. Packing a wound also offers the opportunity to remove detritus material from these spaces through the mechanical action achieved by removing these packing materials. Filling wound dead space will also help to stabi-

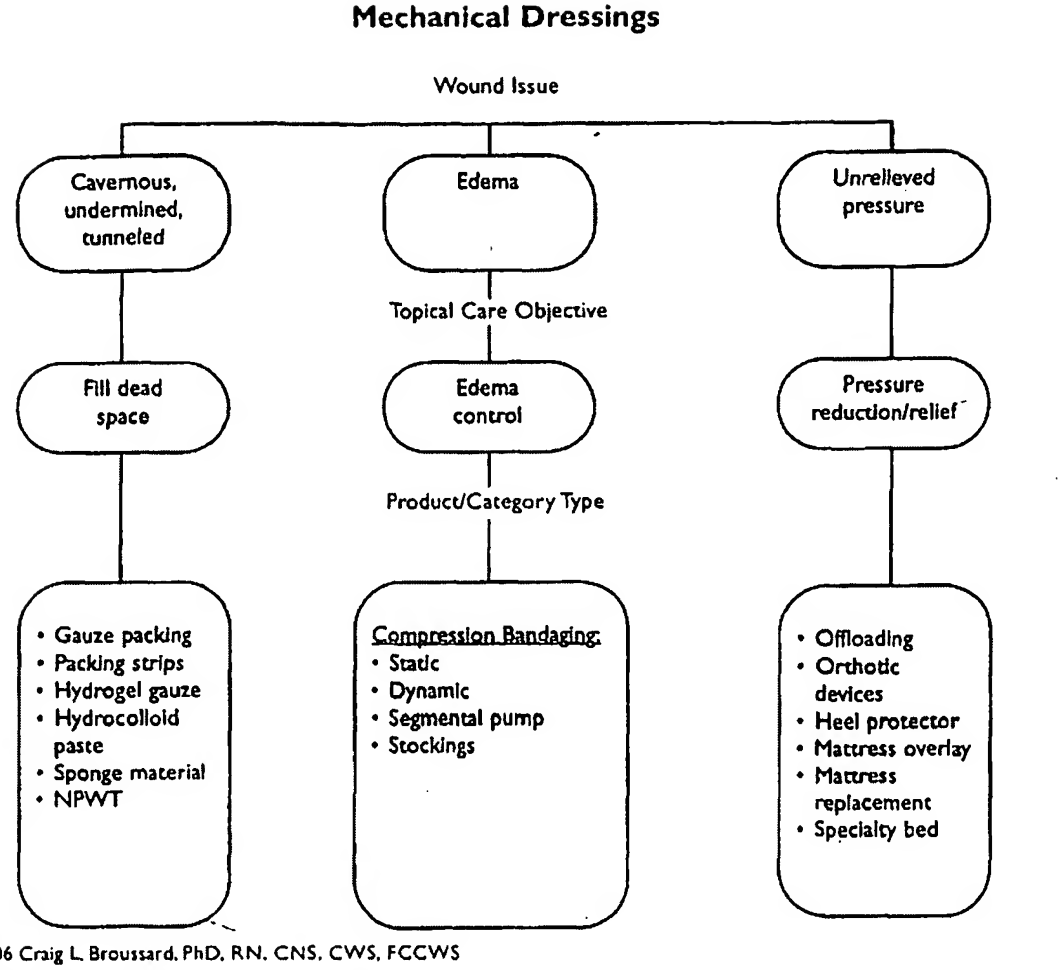

Figure 2. Wound stabilization pressure reduction edema management.

lize the wound and prevent unnecessary movement of the wound. Filling cavernous empty space in a wound can be accomplished with a variety of products. For heavily exudating wounds, alginate and hydrofiber products can accomplish this while absorbing large amounts of drainage. In addition, foam filler products are available in forms that can be placed into a wound cavity. A combination of an alginate or hydrofiber as a filler covered with a foam dressing makes an excellent choice for heavily exudating cavernous wounds. Less exudative cavernous wounds benefit from packing materials that include gauze and packing strips. Hydrocolloid paste offers a method to fill smaller wounds that have little or no exudate and will also fill small amounts of dead space. Hydrocolloid paste may not be as good of a choice for tunneled or undermined wounds as other packing materials. Hydrogel gauze is a good choice for minimally or nonexudating wounds. Damp or moist saline gauze offers another option for packing wounds; however, care should be taken to prevent maceration of the wound.

The use of NPWT offers several advantages to cavernous wounds. Through the unidirectional application of negative pressure, wound stabilization occurs, preventing the wound from unnecessary movement that may decrease wound-healing potential. Negative pressure wound therapy presents an excellent choice in filling dead space in exudating wounds. The facilitation of granulation tissue formation with NPWT will decrease the amount of time needed for a wound to decrease in size and eliminate the need for packing materials. Negative pressure wound therapy may also enhance wound closure in tunneled and undermined wounds from deep within these spaces. One strategy is the use of denser foam materials that are packed less deeply over time to facilitate tissue adherence deep within these spaces. For detailed information on NPWT, see Chapter 30.

Packing strips offer the opportunity to fill empty space while minimizing damage to the wound for undermined

wounds and wounds with narrow openings, such as tunneled wounds. Dry packing strips may be used for exudative wounds. For wounds with minimal or no exudate, strips impregnated with hydrogel or saline are options. Packing materials also offer the opportunity to deliver topical medications and/or disinfectants within the wound. Additional fillers may be needed for wounds that are both cavernous and undermined or tunneled. An appropriate secondary dressing is necessary to complete the dressing. Secondary dressings may include foam for exudating wounds, gauze for less exudating wounds, or a composite dressing that will offer some absorption and perhaps an adhesive border. Care should always be taken to avoid over packing a wound with a filler. Over packing exerts pressure within the wound and can lead to decreased circulation and/or a pressure-related injury within the wound.

Edema management is the second wound issue mechanical dressings address. Edema management is the mainstay of care for the person with a venous ulcer. These wound will fail to heal without edema management. While other surgical interventions are available, it is the dressing that helps to promote immediate edema control. Two dressing types are available in the management of edema during wound healing. These dressings can be classified as either static compression or dynamic or elastic compression. Static compression dressings are often referred to as paste bandages. These dressings stiffen after application. The primary mechanism of action is to assist calf muscle pump action. The bandage itself provides no active compression so that when the patient is inactive, there is no effect with the exception of prevention of excess fluid accumulation in the extremity. These paste systems may be of particular use in persons with dermatological changes associated with venous stasis and lymphedema; however, the process of compression and control of edema may be more important in assisting with resolution of these skin changes. The frequency of static compression bandage change is typically weekly unless excess exudate dictates more frequent change. For detailed information on compression, see Chapter 48.

Dynamic or elastic compression not only assists in calf muscle pump action but also applies continuous compression to the extremity, assisting with edema control regardless of activation of the calf pump. There are several dynamic bandaging systems available. Some systems consist of 4 layers of dressings, while others consist of 3 and some have only 2 layers. Common to all systems is a layer of elastic roll bandaging. Elastic bandaging systems are available latex free for those who are allergic. A primary dressing, such as a calcium alginate, hydrofiber, or foam dressing, may be applied to the wound to assist in exudate management. Foam dressing material may also be used to pad bony prominences to prevent pressure-related injury from the compression bandage. Dynamic bandage systems are generally changed weekly; however, when compression bandaging is first applied, mobilization of large amounts of fluid may necessitate twice weekly bandage changes until edema is better managed, as these bandages will loosen with the lessening edema. The ankle-brachial index (ABI) should be assessed to screen for arterial insufficiency prior to application of a compression system. Once applied, capillary refill should be checked to assess if too much compression has been applied.

Intermittent pneumatic compression devices, sometimes referred to as lymphedema pumps, and gradient compression stockings are other local wound management strategies that can be used to control edema. Intermittent pneumatic compression devices generally are reserved in venous ulcers to wounds that have failed to heal after a 6-month trial of conservative therapy, including compression bandaging. Pneumatic compression devices can be segmented devices or nonsegmented devices. Typically, nonsegmented devices are applied first. If this approach fails, a sequential, segmented compression device may be used. Many limitations relate to the use of pneumatic compression devices, and thorough reimbursement research is necessary when considering the use of these devices.

Gradient compression stockings are primarily used in the prevention of venous ulcers and their recurrence. The premise for the use of these stockings is that they help engorged veins to return to a more normal shape, allowing valves to function more normally, and for the calf muscle pump to be more efficient. The end result is edema control through reduction and prevention of swelling. Gradient compression stockings are rated by the amount of compression they supply. Light compression, or a Class 1 stocking, is generally considered to be between 20 mmHg–30 mmHg and is primarily used for the treatment of varicose veins. Moderate support, Class 2, provides 30 mmHg–40 mmHg pressure and is used to prevent ulcer recurrence. Some practitioners use Class 2 stockings in the treatment of venous ulcers once compression bandaging has adequately controlled edema. Class 3 or high compression stockings provide 40 mmHg–50 mmHg of pressure and are used primarily in the treatment of lymphedema. Some practitioners will use high compression stockings to treat venous ulcers; however, donning these stockings with a primary dressing can be challenging. Very high levels of compression (Class 4) stockings apply 50 mmHg–60 mmHg compression pressure and are used mainly in lymphedema management. Despite the level of compressing pressure, most stockings present the patient with the challenge of applying the stocking. Applying the stocking when first arising in the morning, when edema is reduced from being in a supine position overnight, will make application easier. Providers of stockings have attempted to address this with donning

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devices where the stocking is stretched over a wire frame to facilitate application. Other systems include a slick inner liner that can be removed and makes donning easier. Some systems allow for lower compression stockings to be applied one on top of another, thus facilitating the additive effect of combined compression (ie, two 15 mmHg stockings result in 30 mmHg of compression). Still other methods of application include the use of such home tried strategies as applying the stocking over a plastic bag with handles (eg, a plastic grocery bag), then using the handles to pull the bag from under the stocking. Stocking wearers should have at least 2 pair of stockings so that 1 can be washed and dried while the other is being worn. The life expectancy of a stocking is approximately 3 to 4 months. After that time, stretching of the stocking will result in decreased therapeutic value.

The third aspect of mechanical local wound management strategies is related to reducing the risk of repetitive trauma and sustained pressure on an ulcer site. Pressure reduction and relief strategies can be classified into 2 aspects of offloading that include ambulatory offloading and nonambulatory offloading. Ambulatory offloading is related to the reduction of plantar and lower-extremity ulcer pressures. The classic wound type that would be considered is the plantar surface neuropathic and/or diabetic ulcer. The classic offloading device for a diabetic ulcer has traditionally been the total contact cast. The application of the total contact cast requires training and experience and may not always be an option for wound care providers. Traditionally, the total contact cast was a plaster cast. Fiberglass materials make drying and curing the cast much faster thereby making these materials more desirable. A newer total contact cast kit makes the availability of total contact casting much more user-friendly in wound care clinics and settings where total contact casting has not been an option. An alternative to total contact casting includes walking boots. Outcomes associated with the use of walking boots are comparable to those achieved by total contact casting. An issue with the walking boot is the lack of reimbursement, sometimes making this an unaffordable out-of-pocket expense for many patients. Half-shoes provide another offloading strategy. The idea behind the half-shoe or wedge shoe is that weightbearing occurs from the midfoot to the heel, or midfoot to toes in a heel wound, depending on how the shoe is designed to offload. These do not provide the degree of offloading that total contact casting and walking boots provide; however, they are readily available, inexpensive, and offer more reduction in plantar surface pressure than street shoes. Felt and foam cut to accommodate and offload the wound is another method that is sometimes employed to reduce plantar pressure. A considerable amount of anecdotal evidence suggests that this may be a good offloading strategy; however, no good clinical trials clearly identify this practice as superior to other offloading methods. A newly available offloading device that consists of a combination foam and plastazote insert with removable pegs offers an alternative to offloading plantar surface wounds. In addition to the insert, a sandal-type shoe is available with a removable toe cover to protect toes, keep the dressings clean and dry, and prevent foreign objects from entering the shoe.

An off-the-shelf offloading device is the Integrated Prosthetic and Orthotic System (IPOS). Offloading is similar to the half or wedge shoe but also helps to maintain a degree of dorsiflexion. This device is technically an orthotic device. The heel relief orthosis offers an opportunity for ambulatory offloading of heel ulcers, including diabetic ulcers and pressure ulcers. Other orthotic devices requiring custom creation by an orthotist include the Ankle-Foot Orthosis, the Contracture Reduction Orthotic Walker (CROW), and the Patella Tendon Bearing Brace (PTB). The CROW is particularly useful in people with Charcot deformities. The PTB allows weightbearing to occur below the knee so that no weightbearing occurs on the plantar surface. This has been successfully used in those with transmetatarsal amputations that have subsequently developed plantar ulcers where other offloading techniques have failed. For further information, see Chapter 56.

Nonambulatory offloading is typically related to pressure reduction or relief required in the treatment and prevention of pressure ulcers. Pressure relief surfaces consist of static surfaces and dynamic surfaces. Static surfaces include air, foam, and gel or water mattress overlays. Dynamic surfaces may be low-air-loss or alternating pressure mattresses. Air-fluidized beds offer almost complete offloading. Many devices are available to offload specific body parts, such as heels. These consist of foam blocks, air-filled devices, and splints that cup the heel so that the heel is free from pressure. Similar devices are available for other bony prominences, such as elbows, to prevent pressure ulcer injury. For further information, see Chapter 62.

Activation of the Wound Environment

The third aspect of local wound management and topical dressings seeks to use dressings and products that will cause something to happen in the wound, such as a change in the character or expression of the wound (Figure 3). This category of dressings and local wound management consists of what can be classified as dynamic dressings. These are dressings that enhance the wound through such things as growth factors, stimulate a response in the wound through chemotaxis or other processes, suppress expression of proteases, or reduce the necrotic load on a wound. Products that offer

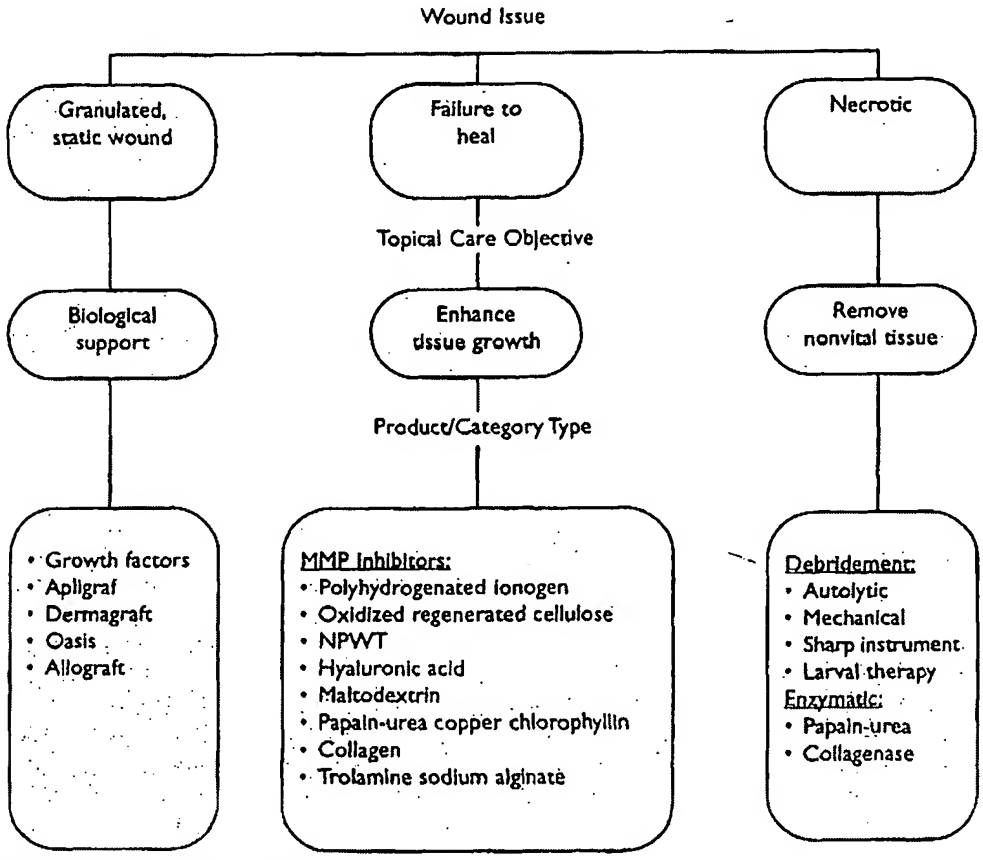

Figure 3. Activation of the wound environment.

biologic support may be beneficial for those wounds that are clean and granulated yet have stopped progressing. The use of growth factors to stimulate the wound as in the case of platelet-derived growth factor (PDGF) may be of benefit. Becaplermin is the commercially available recombinant DNA form of PDGF-bb used in diabetic foot ulcers.

Allograft products, such as cadaveric skin, offer a temporary closure solution for wounds. Noncadaver allograft products are available in acellular and cellular forms. Acellular forms may include type I bovine collagen with a silicone sheeting as seen in the product Integra® (Integra LifeSciences, Plainsboro, NJ). Other acellular allograft products include AlloDerm (LifeCell Corp, Branchburg, NJ) and Graftjacket® (Wright Medical Technology, Arlington, Tenn). One cellular allograft is Dermagraft® (Advanced BioHealing, La Jolla, Calif), a cryopreserved bioabsorbable mesh framework seeded with metabolically active fibroblasts derived from neonatal foreskin. It is indicated for use in the treatment of full-thickness diabetic ulcers. Application of this product is weekly for up to 8 applications. A secondary, nonadherent dressing is required. Apligraf™ (Organogenesis, Canton, Mass) is a living bilayered cell therapy. It is indicated for the treatment of venous ulcers and diabetic neuropathic ulcers. A nonadherent dressing covering the product is necessary to prevent removal of the product during dressing changes. The silicone-based products are particularly useful with Apligraf. Dressing changes for the first week following application of this product should be restricted to secondary bandaging so as to not disturb this product. Subsequently, routine dressing changes may resume. A third product to activate the wound environment is another acellular product. Oasis® (Healthpoint, Fort Worth, Tex) is a small intestinal submucosa product that contains an extracellular collagen matrix that acts as a scaffold for tissue growth. This product requires an appropriate secondary dressing. This product may be applied weekly. Failure to promote healing by all of these products is

closely related to wound preparation. Wounds must be free of necrotic tissue; therefore, sharp instrument debridement prior to application is highly recommended. In addition, wounds that are contaminated, colonized, or infected will have poor outcomes associated with these products. Good bacterial control of the wound is necessary.

A second group of products that serves to activate the wound environment is aimed at those wounds that are failing to heal despite efforts to alleviate the underlying cause of wound healing failure. For example, an ischemic wound continues to fail to heal despite a revascularization procedure. For this wound, the primary objective of topical products is to enhance tissue growth. These products can be subdivided into those products that stimulate something to occur and those that inhibit a process in the wound. Some of these products are used atypically. For example, an enzymatic debriding agent that contains copper chlorophyllin enhances granulation tissue formation. The mechanism of action promoting granulation tissue is probably in the copper chlorophyllin's ability to reduce fibrin formation allowing macrophages to move into the wounded area, which signals fibroblasts to initiate collagen deposition. Copper chlorophyllin further enhances the structural integrity of this new collagen matrix. Papain-urea also helps to keep the wound free of necrotic tissue. Applying this product to the wound is simplified by placing the product on the secondary dressing instead of attempting to apply it directly to the wound surface. Using this method ensures distribution of the product across the entire wound surface.

Several products act as chemoattractants to either macrophages or fibroblasts. The use of these products can stimulate and enhance wound healing in select wounds. For example, a wound that is failing to exhibit any inflammatory response may benefit from the chemoattractant effect on macrophages to "kick start" the wound into a pattern of healing. A product that offers wound stimulation through macrophage chemotaxis is Multidex® (DeRoyal, Powell, Tenn), a maltodextrin hydrophilic wound dressing. This product is a high molecular-weight polysaccharide that acts as a chemoattractant for macrophages when applied to the wound. The product is available in a powder for wounds that have exudate. When the product comes in contact with wound exudate, it absorbs the exudate and turns into a gel. It also is available in a hydrogel form for wounds that are dry or have minimal exudate. In addition to serving as a chemoattractant, this product effectively lowers wound pH, making the wound environment more hostile to bacteria. It is also an effective odor-controlling product. A secondary dressing needs to be used with this product. Dressings typically are changed daily to every other day. Biafine, a trolamine sodium alginate, is another macrophage chemoattractant. Biafine promotes a moist wound environment. In exudating wounds, a secondary, absorptive dressing is needed. For undermined or tunneled wounds, gauze or packing strips can be impregnated with the product for delivery into these areas. A secondary dressing is required, and dressings should be changed daily. One hyaluronic acid dressing, Hyalofill® (ConvaTec, Princeton, NJ), is supplied in both a fleece sheet and rope form. The proposed method of action for this dressing is the facilitation of movement or chemotaxis of fibroblasts to the wound area. Hyaluronic acid aids in moisture control by absorbing exudate and forming a gel. A secondary dressing is required. This dressing is usually changed daily.

Negative pressure wound therapy in the form of vacuum-assisted closure (V.A.C.™, KCI, San Antonio, Tex) foam technology offers a unique method of stimulating wounds to progress. Evidence suggests that the application of mechanical force to the wound and the foam strut spacing induces tissue deformation at the level of individual cells. This results in a stretching of the individual cells that induces cell proliferation, angiogenesis, and subsequent wound healing. It is suggested that these micromechanical forces may be the most significant mechanism of action of V.A.C. Therapy. See Chapter 30 for further information.

A final group of dressings that cause a change in the wound environment includes those dressings that inhibit matrix metalloproteases (MMPs). Two product types that inhibit proteases are available. One product is a freeze-dried bioresorbable collagen and oxidized regenerated cellulose product. As exudate containing the MMPs is absorbed by this product it turns into a gel. Growth factors are protected from degradation through binding and inactivation. This product may be moistened with saline if the ulcer is dry or nonexudating. A secondary dressing is needed. Dressing change is typically every 2 to 3 days. A second protease inhibitor consisting of a polyhydraged ionogen consisting of rubidium chloride, potassium chloride, and sodium chloride is believed to work by suppressing expression of MMPs. This dressing is an impregnated gauze dressing. A secondary dressing is necessary. For exudating wounds, an appropriate absorptive dressing may be used. This dressing is usually changed daily.

Dynamic dressings and topical wound management strategies offer the clinician unique opportunities to influence wound healing. With an understanding of both normal wound healing and chronic wound healing, these strategies not only can improve wound outcomes but also can decrease time to healing, effectively decrease the potential of complications, and increase morbidity. These strategies should be considered in conjunction with passive wound care strategies aimed at optimizing the moist wound environment.

Control of Infection/Bioburden

Another objective of topical dressings and local wound

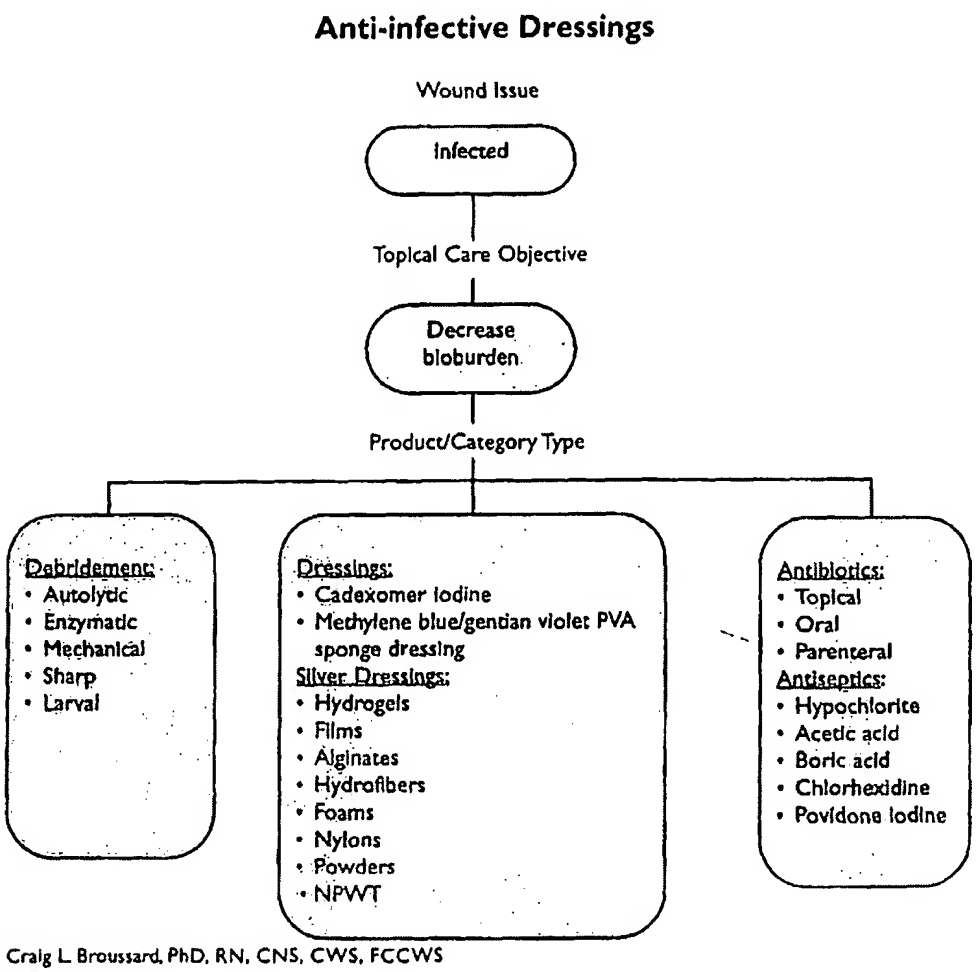

Figure 4. Control of infection/bioburden.

management is to decrease bioburden and control wound infection (Figure 4). Wounds contaminated and/or colonized with bacteria can also have negative outcomes. Wounds generally have a central zone of hypoxia. Bacteria competing for available oxygen may increase this hypoxia. Topical management of wounds can decrease bioburden in wounds.

One of the most important aspects of topical wound management in the control of bioburden is debridement. While sharp instrument debridement offers the quickest and most reliable method of wound cleaning, it may not always be the most suitable. For example, one would want to know arterial status prior to performing an extensive debridement. Other methods of debridement include autolytic debridement, enzymatic debridement, mechanical debridement, and larval debridement.

Autolytic debridement occurs in a moist wound healing environment. Dressings that maintain the moist wound environment facilitate autolytic debridement. Avascular, necrotic tissue is rehydrated. In addition, certain dressings help to sequester white cells in wound fluid at the wound site allowing autolysis of necrotic tissue to occur. Dressings that facilitate autolytic debridement include films, hydrocolloids, and hydrogels. Foams, alginates, and gauze dressings also promote autolysis if hydrated. Dry eschar will need to be cross-hatched to be adequately hydrated for autolysis to occur. Dressings may need to be changed more frequently so that the autolytic process can be assessed and monitored.

Collagenase and papain-urea are enzymatic debriding agents. Collagenase degrades collagen fibers. Papain-urea degrades cysteine amino acid residue. Both are selective to their respective target and offer effective means to clean wounds. These products are considered pharmaceuticals and require a physician prescription. Both require a secondary dressing. The process of applying the product to a secondary dressing then applying the dressing to the wound facilitates

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application. While both products are considered to be safe on healthy tissue, care should be taken to avoid applying product to the periwound area. Dressing changes usually occur daily.

Mechanical debridement can be provided in several ways. Wound irrigation is the most commonly used form of mechanical debridement and should be done at each dressing change to remove the normal accumulation of wound detritus and bioburden. The use of normal saline in a 35-mL syringe with a 19-gauge needle is the most described method of wound irrigation. Other prepackaged irrigation systems are available. For example, more aggressive irrigation for more adherent necrotic tissue can be achieved with pulsed lavage systems. Whirlpool, while out of favor in wound care, offers an opportunity to remove large amounts of debris from a wound. One dressing product provides a rinsing action while applied to the wound. TenderWet Active (Medline, Mundelein, Ill) uses Ringer's solution to contribute sodium, potassium, and calcium to the wound bed. The action of the dressing allows for absorption of pathogens, debris, and necrotic tissue into the dressing. TenderWet Active requires a secondary dressing. This dressing should be changed daily.

The use of whirlpool should be limited in use and duration, as damage to healthy tissue and maceration of surrounding tissue are common. A dressing choice for mechanical debridement is the wet-to-dry dressing. The use of wet-to-dry dressings for debridement should be "retired" from modern wound care, as other methods of debridement are more selective and less traumatic and painful for the patient. The most selective, rapid, and certain way to clean a wound is through sharp instrument debridement. A final debridement method involves the use of fly larva or maggots.

Bioburden control also can be achieved through the use of dressings. Three dressing types are available to assist the clinician to this end. Perhaps the most well known class of dressings are silver dressings. Three types of silver forms are available in dressing materials: colloidal, ionic, and nanocrystalline. The major difference in these silver types is the duration of the rate of release and subsequent availability of silver in the ulcer. This will determine frequency of dressing change. Silver acts as a microbial barrier. In the presence of exudate, silver ionizes and releases into the wound. Fortunately, silver is available in dressing materials that are similar to the passive dressings. Silver alginate and silver foam products can be used with exudating wounds. Silver films can be used on nonexudating wounds. Silver gels can be used on nondraining or dry, desiccated wounds. In essence, the choice of silver product should mimic the choice of substrate dressing that also would optimize the moist wound environment. Dressing change regimens for silver dressings are predicated on the form of silver incorporated in the dressing. Nanocrystalline silver has the slowest release of silver. Some formulations of nanocrystalline silver may only require a dressing change every 7 days. Colloidal silver may need reapplication every 2 to 3 days. Ionic forms of silver are rapidly absorbed and may need daily reapplication.

Another modern dressing for the control of bacteria is cadexomer iodine. This product contains elemental iodine in hydrophilic beads. As the beads absorb wound exudate iodine is released into the wound. Bacteria, enzymes, and cellular residue also are absorbed into the hydrophilic beads and help cleanse the wound. Cadexomer iodine is available in 2 forms: Iodoflex™ (Smith & Nephew, Largo, Fla), a gel pad with absorption capability, and Iodosorb™ (Smith & Nephew, Largo, Fla), a gel form that can contribute moisture to a minimally exudating or dry wound. The release of iodine determines dressing change. As iodine is released, the dressing changes color from a reddish brown to yellow. This color change indicates that the dressing needs to be changed. Even so, this dressing should be changed every 2 to 3 days. A secondary dressing is necessary. An absorptive dressing may be necessary in heavily exudating wounds. Cadexomer iodine is contraindicated in persons with iodine sensitivity. It should be avoided in persons with thyroid dysfunction and impaired renal function.

A third form of antimicrobial dressing benefits from the use of methylene blue and gentian violet in a sponge form known as Hydrofera Blue (Hydrofera LLC, Willimantic, Conn). This product offers a new topical dressing approach to infected and colonized wounds. While these pigments have been used for many years, only recently have they been incorporated into a sponge format that allows for absorption of exudate while providing bacterial control. This dressing must be moistened with saline prior to application to the wound. A secondary dressing is necessary. Dressing change regimens range from daily to every 3 days. Change in color from blue to white indicates the need for a dressing change.

A fourth dressing strategy available for control of bioburden is NPWT. This topical dressing strategy increases circulation and oxygenation to the wound. With increased oxygen there is an increased resistance to infection and enhanced oxidative bacterial killing. In addition, the mechanical suction of NPWT removes bacteria from the wound. Foam technology combined with silver technology that further improves control of bioburden is available.

Another local wound management technique to control bioburden is considered controversial in wound care. This consists of the use of antiseptics. Antiseptics can be cytotoxic, particularly to fibroblasts. The decision to use antiseptics involves a risk/benefit decision. A couple of well placed questions can guide in this decision. Is the bioburden or infection present in a wound causing more damage than the

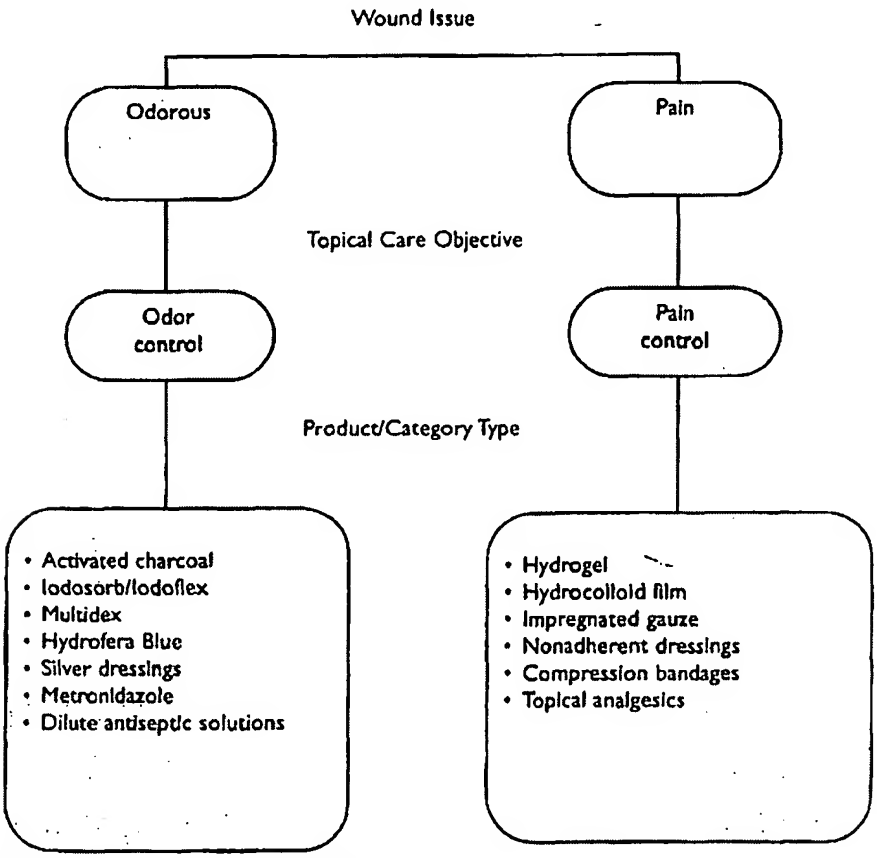

Figure 5. Quality-of-life improvement.

cytotoxic agent? Are other topical products that are less cytotoxic available to reduce bioburden? Are other systemic interventions more appropriate? There is a place for antiseptics in wound care; however, it is inherent that the clinician use sound clinical judgment in this decision. Know when to stop using these products. The use of antiseptics should be very short term. Antiseptics used in wound care include povidone-iodine, sodium hypochlorite solution or Dakin's Solution, acetic acid solution, or Domboro's solution, boric acid solution, chlorhexidine, and hydrogen peroxide. For further information, see Chapter 34.

Quality-of-Life Improvement

The final class of dressings and topical wound management strategies are comfort dressings aimed at improving quality of life (Figure 5). The main objectives of these strategies include odor control and the reduction of pain associated with a wound. Wound odor can seriously impact quality of life. It can prevent a person from experiencing meaningful social interactions and can limit the person's ability to be in public. There are several strategies that can help to eliminate or significantly reduce wound odor.

First, one must determine the origin of wound odor. Is the odor a result of infection? If so, treatment of the infection should reduce or eliminate the odor. The use of systemic antibiotics may be necessary; however, the use of topical antimicrobial dressings can be effective in reducing and eliminating odor. These dressings include cadexomer iodine or Iodosorb/Iodoflex, Multidex, Hydrofera Blue, and silver-based dressings. In addition, the use of dilute antiseptic solutions may also be of benefit not only in controlling topical odor-causing bacteria but also by serving as a deodorizer. If odor is the result of the breakdown of necrotic tissue, the removal of the necrotic tissue through debridement should be performed.

One dressing approach to odor control is the use of acti-

vated charcoal dressings. These dressings serve as a filter to trap odor. Charcoal dressings are available in 2 types. One type includes activated charcoal combined with another dressing type, such as silver or foam. These dressings have dual purposes including control of bacteria or absorption of exudate as well as odor control. A second type is simply a charcoal pad that can be placed over other dressings materials to serve as a filter for volatile odors. Depending on the type of product, dressing change may be every 2 to 3 days to as infrequently as every 7 days. Activated charcoal tends to loose its efficacy when it becomes moist with exudate. A charcoal pad used as a filter on top of other dressings may be reused if it does not become contaminated with exudate. Other measures to control odor in the immediate environment of a person with an odorous wound include commercially available room deodorants and such simple measures as placement of a saucer of vinegar in the room. One method that has been used successfully in home environments includes the use of cat litter placed in strategic places in a room to help absorb odors.

A particularly challenging odorous wound type is the fungating cancerous lesion. In addition to the use of dilute topical antiseptics, such as hypochlorite and acetic acid, the use of metronidazole may be beneficial. Metronidazole, an antifungal, may be given systemically, but due to potential side effects, it may be better to apply it topically to the wound. Metronidazole is available in gel formulation. It has also been applied by sprinkling the contents of a capsule directly to the wound surface. The primary mode of action for odor control is in the bactericidal capability of this drug. Treatment with metronidazole is generally topical application daily for 1–5 days. An appropriate secondary dressing to manage exudate is generally required.

The second objective to improve quality of life with topical strategies includes the selection of dressings that either help alleviate pain or are less likely to inflict pain when removed. Amorphous and formed hydrogel dressings are soothing to painful wounds. Hydrocolloid dressings and impregnated gauze dressings are also soothing dressings. These dressing types are easy to remove and are less likely to cause pain than dressings that may absorb too much exudate and adhere to the wound surface. Nonadherent dressings and those dressings with petrolatum or silicone are less likely to adhere to the wound, making them desirable for use with partial-thickness wounds, which tend to be more painful than full-thickness wounds. Compression bandaging to control edema will help to reduce the aching associated with venous leg ulcers as well as lymphedema. The use of topical analgesics may be necessary during dressing changes. Lidocaine solution applied to soak a dressing prior to removal can be effective. The use of analgesic gels prior to debridement should always be considered and may be of benefit prior to reapplication of a dressing. For further information, see Chapter 11.

Conclusion

In today's environment of modern topical dressings, numerous choices that optimize the local wound environment to facilitate wound closure are available to the clinician. A number of pitfalls can impede the clinician. The "dressing *du jour*" attitude that "this dressing worked on this patient therefore it will work on all patients" limits clinical decision making. There is no one perfect dressing. It is also easy to become enamored with a particular manufacturer and not seek dressings that may not be part of a particular manufacturer's offering. In addition, as clinicians, we are often bound by a particular institutional buying contract, making user-preferred dressings unavailable. This may also be the case when an institution changes from one buying group to another. That does not necessarily preclude being able to go off contract for unique products that may not be available through a particular buying group. Outside the United States, clinicians may be faced with their country's particular formulary that may limit the availability of products.

Only one approach to selecting topical products is offered here. Others decision strategies are available, and the wise clinician would learn from multiple strategies to enhance his or her decision-making ability. This approach was born from clinical practice and observing product performance. It may not reflect every possible dressing available. Dressing products are ever evolving and new products become available. The wise clinician always circles back to the underlying etiology of why a wound is slow to heal or is failing to heal. It is this understanding that enhances the ultimate outcome. The choice of dressing or topical wound management strategy will enhance the potential to heal but will not in itself cause a wound to heal.

Take Home Messages for Practice

- Identify and correct the underlying cause of wound healing failure.
- Use topical wound management strategies to enhance the host's wound healing ability.
- There is no one magic topical wound management strategy.
- Always keep the patient in focus when choosing topical strategies.

Self-Assessment Questions

1. The category of passive dressings aims to accomplish what wound care objective?

A. Optimization of the moist wound environment

B. Wound manipulation, pressure reduction, or edema management
C. Activation of the wound environment
D. Control of infection and bioburden

2. Optimizing the moist wound environment would include which of the following dressings?
A. Compression bandaging
B. Matrix metalloprotease inhibitors
C. Calcium alginates
D. Charcoal

3. The choice of a silver-based dressing should include what objective aside from control of infection or bioburden?
A. Odor control
B. Pressure reduction
C. Biological support
D. Absorb drainage

Answers: 1-A, 2-C, 3-A

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Suggested Reading

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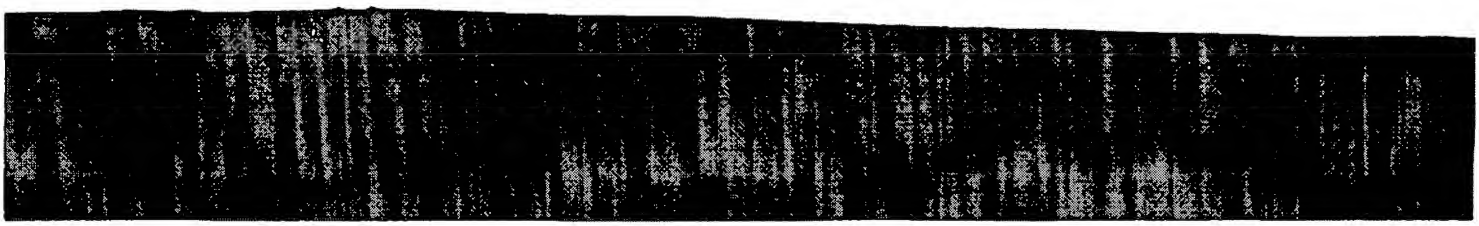

Pressure Ulcer Evaluation Case Series of 18 Wounds

Evaluation of XCell® Cellulose Wound Dressing on Wound Healing of Pressure Ulcers

Marie Brown-Etris, RN, CWOCN
Marion Punchello, LPN
Etris Associates Inc., Philadelphia, PA

ABSTRACT

As part of a study of 27 patients (33 wounds) evaluating the effectiveness of XCell Cellulose Wound Dressing, a subset of 15 patients (18 wounds) had Stage II to Stage IV pressure ulcers. Of the evaluable pressure ulcers, 14 demonstrated improvement or healing of the ulcer. Results demonstrated that XCell promoted autolytic debridement of necrotic slough and eschar by maintaining a moist wound environment. In addition, pressure ulcers with XCell had increased granulation tissue that was vibrant red in color. In general, the wounds decreased in size over the study period by an average of 39% and in one case healing was observed. XCell can be used effectively on Stage II to IV pressure ulcers.

Evaluation of XCell® Cellulose Wound Dressing on Wound Healing of Pressure Ulcers

Marie Brown-Etris, RN, CWOCN
Marion Punchello, LPN
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INTRODUCTION

Ulcerated wounds resulting from vascular, metabolic or physical trauma are frequently the most recalcitrant to therapeutic intervention. Although more than one etiologic factor can contribute to the pathogenesis of an ulcerated wound, most are likely due to a single precipitating event leading to ischemic and tissue necrosis. Pressure ulcers, for instance, are precipitated by prolonged excessive pressure to tissues over bone.

The science of wound healing has advanced rapidly since George Winter described the advantages of moist wound healing in 1970. The medical profession has come to recognize that the optimum environment for the wound bed is moist and not dry. Wound bed preparation is the key to successful wound management.[1] From this the following guidelines for the use of wound dressings has been suggested:

1) Use a dressing that will maintain a moist wound environment.
2) Use clinical judgment when selecting a moist wound dressing.
3) Choose a dressing that will keep the peri-wound skin dry while maintaining the moisture within the wound.
4) Use a dressing that will control wound exudate without leading to desiccation of the wound bed.
5) Use dressings that are easy to apply and do not require frequent changes.
6) Fill cavities to avoid impaired healing and increased bacterial invasion.

XCell® Cellulose Wound Dressing is a unique biomaterial composed of biosynthesized cellulose. It is distinguished from plant-derived cellulose by its high hydrophilicity and a multi-layered three dimensional laminar structure. It has been bioengineered to both donate fluid and absorb liquid, depending on the moisture content of the surface the dressing contacts.

A study of 33 wounds on 27 patients was conducted to evaluate the effectiveness of XCell on a variety of acute and chronic wounds. Of the 33 wounds, 18 were Stage II to IV pressure ulcers. This article examines the effectiveness of XCell on the pressure ulcer subset.

[1] Schultz G.S., et al.: Wound Rep Reg 2003; 11:1-28.

OBJECTIVE

The objective of this study was to evaluate the performance characteristics and effectiveness of XCell® Cellulose Wound Dressing on Stage II - IV pressure ulcers and to determine the desired technique for successfully treating such wounds.

MATERIALS AND METHODS

XCell Cellulose Wound Dressing (Xylos Corp., Langhorne, PA) was provided as a 3.5in x 3.5in sterile pad. Depending on the site and wound condition, the dressing was either left intact or cut to the size of the wound.

Secondary dressings were placed over XCell and included Alldress™ (Mölnlycke, Göteborg, Sweden), BlisterFilm® (Kendall, Mansfield, MA), Bioclusive® and Kling™ (Johnson & Johnson Medical, Arlington, TX) or 4in x 4in gauze.

STUDY DESIGN

This study was performed as a single center, open enrollment evaluation of XCell® Cellulose Wound Dressing. After Institutional Review Board Approval (St. David's HRRB, Philadelphia, PA), twenty-seven participants, 18 years of age or older with one or more draining wounds involving the dermis, subcutaneous tissue or muscle (with or without bone exposure) were entered into the evaluation. Each participant was observed on a frequency ranging from daily to weekly depending on the type of wound and phase of wound healing. For example, wounds that were heavily draining typically required a minimum of daily dressing changes, whereas those that were epithelializing typically required weekly dressing changes. Patient participation was for up to eight weeks. At a minimum, on a weekly basis, the dressing was changed for a thorough wound assessment, at which time various data were recorded. In addition to completing the case report forms, photographic slides were taken at each visit.

The inclusion criteria for this clinical study were quite broad so that a determination could be made as to where XCell demonstrated maximum performance. For this pressure ulcer subset (18 ulcers), participants were permitted to have a stage II, III or IV pressure ulcer. The effectiveness of this product was based upon comparison of the base line wound condition data to that of subsequent follow-up visits.

RESULTS AND DISCUSSION

Overall, XCell was found to be very effective for use on pressure ulcers. Specific observations included:

- Reduction in wound size within 8 weeks by an average of 39%
- Reduction in height of hypergranulation tissue
- Reduction of necrotic slough and eschar through autolytic debridement
- Increased and vibrant granulation tissue
- Non-adherence to the wound bed

Eighteen full thickness pressure ulcers were evaluated during the study. Of these, two were considered un-evaluable due to non-compliance (1) and a non-product adverse event at one week (1). By the end of the eight weeks, 14 of the 16 remaining wounds demonstrated improvement including one that healed (Table 1, Figure 1). Seven full thickness pressure ulcers entered the study with a 100% granulation tissue base. Three maintained their 100% granulation tissue base until study conclusion while one pressure ulcer decreased granulation tissue base from 100% to 75% which subsequently increased and the wound went on to heal (Figures 2 - 5). Two pressure ulcers reduced from 100% to 80% in granulation tissue and one pressure ulcer lost a significant amount of granulation tissue which was attributed to a wound infection.

Pressure Ulcer Evaluation Case Series of 18 Wounds

Figure 1: Graph demonstrating the average size of the wound (assuming elliptical shape) at the initial examination and at the end of the study. XCell demonstrated a 39% average reduction in wound size over eight weeks.

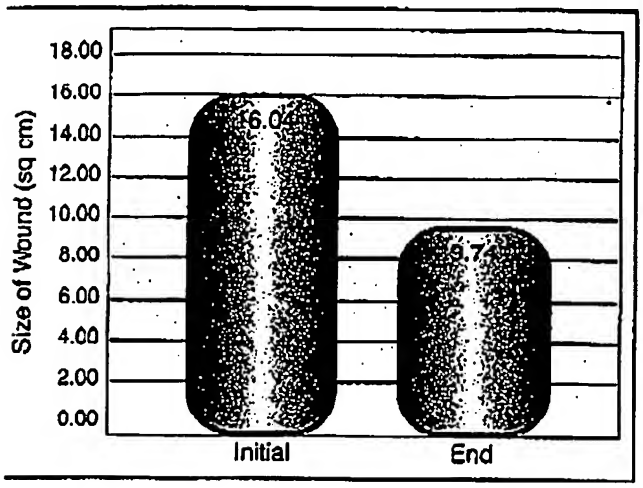

Table 1: PRESSURE ULCER HEALING RESPONSE TO XCELL

Ulcer Type	Healed	Improved	No Change	Unevaluable
STAGE II	—	2	—	—
STAGE III	1	3	—	1
STAGE IV	—	8	2	1

Figures 2 - 5: Healing of a full thickness Stage III pressure ulcer on the heel in a 90-year-old female. Figure 2 demonstrates the wound at the initial visit and shows necrotic tissue. The wound initially measured 1.6cm by 1.1cm. By week 2 (Figures 3 and 4) XCell had facilitated autolytic debridement and epithelialization so that the wound now measured 1.2cm by 1.0cm. Figure 5 shows the wound at week seven measuring 0.5cm by 0.4cm and on its way to healing at eight weeks.

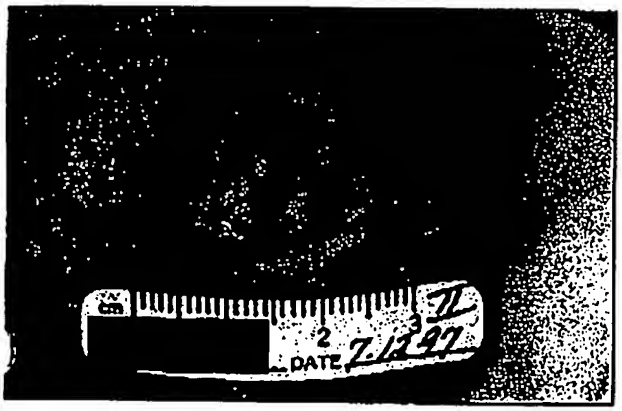

Fig. 2: Initial visit

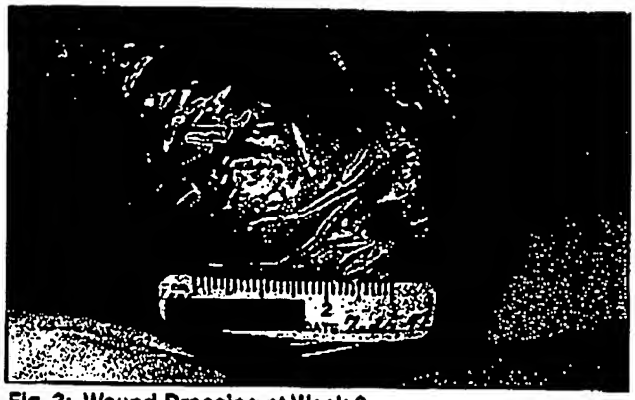

Fig. 3: Wound Dressing at Week 2

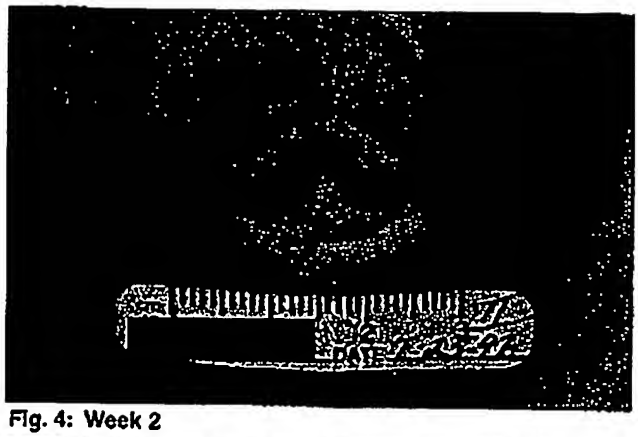

Fig. 4: Week 2

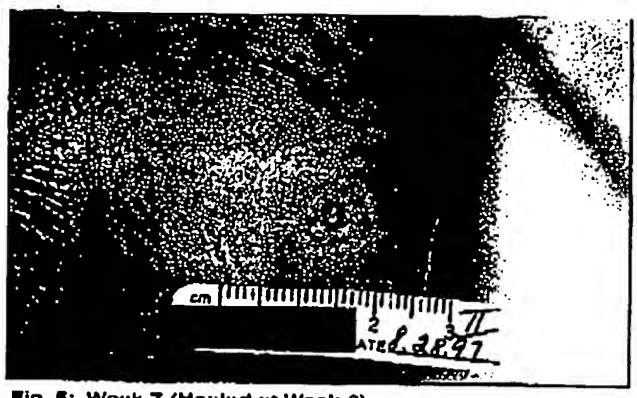

Fig. 5: Week 7 (Healed at Week 8)

As seen in Figures 2 and 4 autolytic debridement was demonstrated using XCell with the removal of slough necrosis. Eight full thickness ulcers entered the clinical study with some percentage of necrotic slough. During the course of the eight-week study, six of those wounds demonstrated a conversion of slough to 100% granulation tissue base (Figures 6 - 8 and 9 -11). When placed on a wound, XCell remains moist allowing the endogenous enzymes to autolytically debride the wound.

Figures 6 - 8: Autolytic debridement of yellow slough in a 50-year-old male presented with a full thickness Stage IV pressure ulcer on his left hip. It was classified as a new wound that had lasted between four and six months and was approximately 70% granulation tissue and 30% necrotic slough. Treatments prior to use of XCell included chemical and autolytic debridement and use of other wound dressings. Figure 6 demonstrates some slough remaining after the first week on XCell. The slough was removed with the XCell dressing (Figure 7) and by week 8 had become 100% vibrant granulation tissue (Figure 8).

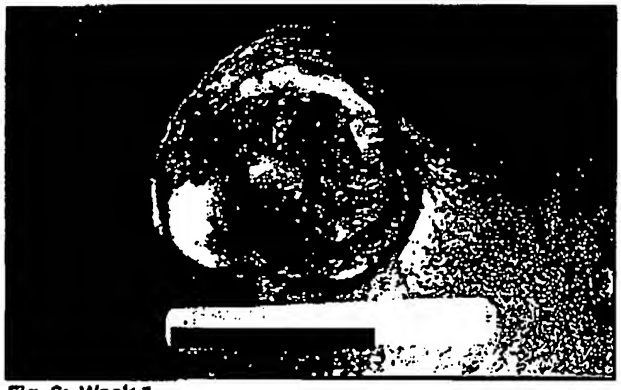

Fig. 6: Week 1

Fig. 7: Removal of Slough into XCell

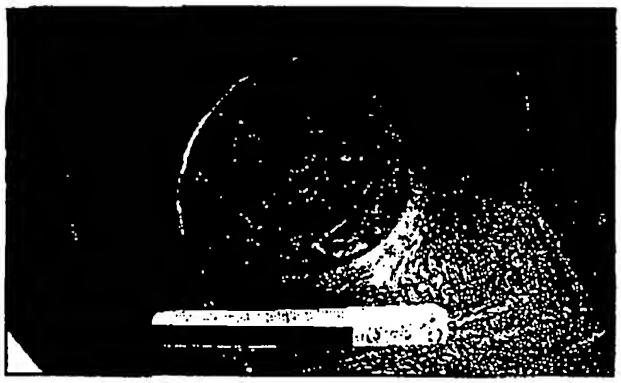

Fig. 8: Week 8 vibrant granulation tissue

Figures 9 - 11: Autolytic debridement of necrotic slough and eschar in a 96-year-old female presented with a full thickness Stage IV pressure ulcer on her sacrum. It was classified as a non-responsive wound that had lasted between four and six months. It was full thickness with approximately 50% granulation tissue, 40% necrotic slough and 10% eschar. Treatments prior to use of XCell included mechanical, chemical and autolytic debridement, and use of other wound dressings. Figure 9 demonstrates that after one week the eschar was gone and the necrotic tissue had decreased. Continued application of XCell resulted in 100% granulation tissue by Week 2 (Figure 10) with the dressing taking up the slough as it was digested by the endogenous enzymes (Figure 11).

Fig. 9: Week 1

Fig. 10: Week 2

Fig. 11: XCell dressing after treatment

Pressure Ulcer Evaluation Case Series of 18 Wounds

A clinical observation made throughout the course of the study was the vibrancy of the granulation tissue base after being treated with the cellulose wound dressing (Figure 12). Figures 13 - 16 illustrate reduction in size of a pressure ulcer on the ankle over the eight-week study, as well as the periwound drying out of a dressing when placed on a wound that was not exudating. The wound remains moist under the dressing.

Anecdotally, XCell appeared to reduce hypergranulation tissue up to the level of surrounding epithelium. This was observed in a wound that presented with this tissue at initial visit.

Figure 12: Illustration of the vibrancy of the granulation tissue after eight weeks of use of XCell in a 66-year-old female that presented with a full thickness Stage IV pressure ulcer on her sacrum. It was classified as a non-responsive wound that had lasted between four and six months. Initially the patient had approximately 85% granulation tissue and 15% necrotic slough. Previous treatments included mechanical, chemical and surgical debridement, and use of other wound dressings.

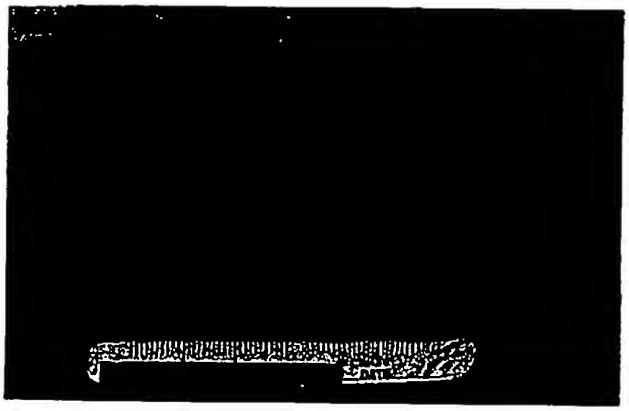

Fig. 12

Figures 13 - 15: Healing of a full thickness Stage III pressure ulcer on the right lateral ankle in a 50-year-old male. The wound was classified as non-responsive and had lasted between four and six months. It had approximately 50% granulation tissue and 50% necrotic slough. Treatments prior to use of XCell included chemical and autolytic debridement and use of other wound dressings. Figure 13 shows the wound after one week of XCell use and the removal of necrotic slough into the dressing (Figure 14). By the end of the study at Week 8, the wound had decreased in size from 11.0sq cm to 5.0sq cm and epithelialization was evident (Figure 15).

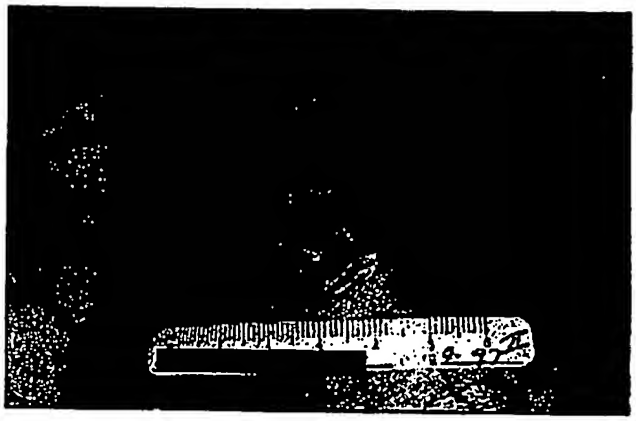

Fig. 13: Week 1

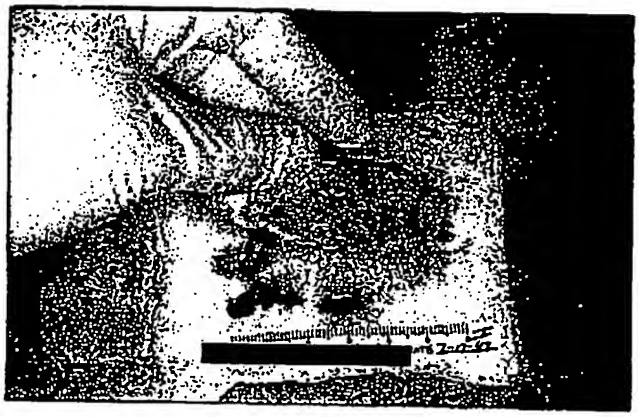

Fig. 14: Dressing after removal

Fig. 15: Week 8

SUMMARY

XCell® Cellulose Wound Dressing performed well in the healing of pressure ulcers. It was able to maintain a moist wound environment during the application period, did not adhere to the wound, and was easy to use.

The most significant wound healing response noted with the use of XCell was the elimination and/or reduction of slough necrosis in full thickness pressure ulcers through the maintenance of a moist wound bed. Application of XCell also reduced hypergranulation tissue to the level of the surrounding epithelium in one pressure ulcer that presented the problem. Of the 16 evaluable wounds in the pressure ulcer subset, 14 showed healing or improvement over the eight-week treatment.

The selection of the appropriate secondary dressing is dependent upon the condition of the wound. This study revealed that wounds with greater exudate would benefit from less occlusive, higher absorptive materials. As well, such wounds may require more frequent dressing changes. For lower exudating wounds, a semiocclusive or occlusive dressing will maintain the moisture level of the dressing and wound.

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Venous Stasis Ulcer Evaluation Case Series of 13 Wounds

Evaluation of XCell® Cellulose Wound Dressing on Wound Healing of Venous Stasis Ulcers

Marie Brown-Etris, RN, CWOCN
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ABSTRACT

As part of a study of 27 patients (33 wounds) evaluating the effectiveness of XCell Cellulose Wound Dressing, a subset of 10 patients (13 wounds) had full or partial thickness venous stasis ulcers. Of the 13 wounds all improved with seven healing during the eight-week study. Results demonstrated that the XCell promoted autolytic debridement of necrotic slough and eschar by maintaining a moist wound environment. Epithelialization was evident and the excellent outcomes resulted in a standard technique for venous ulcers that includes XCell.

Evaluation of XCell® Cellulose Wound Dressing on Wound Healing of Venous Stasis Ulcers

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INTRODUCTION

Chronic wounds are defined by their deviation from the expected sequence of repair and represent a problem for both the patient and the health care provider. Venous stasis ulcers are one etiologic class of chronic wounds that result from venous insufficiency. Improving this insufficiency and/or reducing venous hypertension are paramount. After treatment of the underlying pathology, the wounds require wound bed preparation to stimulate the healing process.[1]

The process of wound bed preparation includes the removal of necrotic tissue, management of exudate and resolving bacterial imbalance.[1] The optimum environment for the wound bed has been demonstrated to be moist and not dry.

As the result of this, a new dressing, XCell Cellulose Wound Dressing has been developed. XCell has been bioengineered to both donate fluid and absorb liquid simultaneously within the various microenvironments of the wound bed depending on the surface the dressing contacts.

A study of 33 wounds on 27 patients was conducted to evaluate the effectiveness of XCell on a variety of acute and chronic wounds. Of the 33 wounds, 13 were full or partial thickness venous stasis ulcers. This article examines the effectivness of XCell on the venous ulcer subset in conjunction with compression therapy.

[1] Schultz G.S., et al.: Wound Rep Reg 2003; 11:1-28.

OBJECTIVE

The objective of this study was to evaluate the performance characteristics and effectiveness of XCell® Cellulose Wound Dressing on venous stasis ulcers. A secondary goal was to develop the optimal technique for treating venous ulcers.

MATERIALS AND METHODS

XCell Cellulose Wound Dressing was provided as a 3.5in x 3.5in sterile pad. Depending on the site, the dressing was either left intact or cut to the size of the wound.

Individual secondary dressings placed over XCell included Alldress™ (Mölnlycke, Göteborg, Sweden), Tegaderm™ (3M Health Care, St. Paul, MN), Hydrasorb™ (Avitar, Canton, MA), or 4in x 4in gauze. Additionally, a stockinette or other compression wrap was used over the secondary dressing.

STUDY DESIGN

This study was performed as a single center, open enrollment evaluation of XCell. After Institutional Review Board Approval (St. David's HRRB, Philadelphia, PA) 27 participants, 18 years of age or older with one or more draining wounds involving the dermis, subcutaneous tissue or muscle (with or without bone exposure) were considered for evaluation of the cellulose wound dressing. Each participant was observed on a frequency ranging from daily to weekly depending on the phase of wound healing. For example, wounds that were heavily draining typically required a minimum of daily dressing changes where as those that were epithelializing typically required weekly dressing changes. Patient participation was for up to eight weeks to evaluate dressing performance and wound healing capabilities. At a minimum, on a weekly basis, the dressing was changed for a thorough wound assessment at which time various data points were recorded. In addition to completing the case report forms, a wound tracing was performed and a photographic slide taken at each visit. Dressings consisted of the XCell Cellulose Wound Dressing and compression therapy.

The inclusion criteria for this clinical study were quite broad so that a determination could be made as to where the cellulose wound dressing demonstrated maximum performance. In the venous ulcer subset (13 wounds), participants were permitted to have a partial or full thickness leg ulcer. The effectiveness of this product was based upon comparison of the base line wound condition data to subsequent follow-up visits and final study day data analysis.

Figures 1 & 2: Dressing placement on ulcer and compression wrap

Fig. 1: XCell on wounds

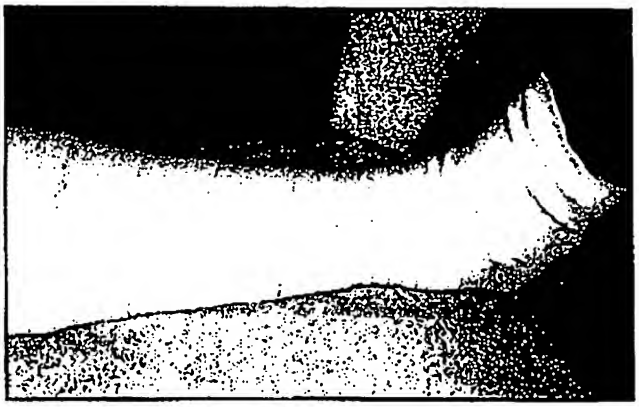


Fig. 2: Compression wrap

/enous Stasis Ulcer Evaluation Case Series of 13 Wounds

RESULTS AND DISCUSSION

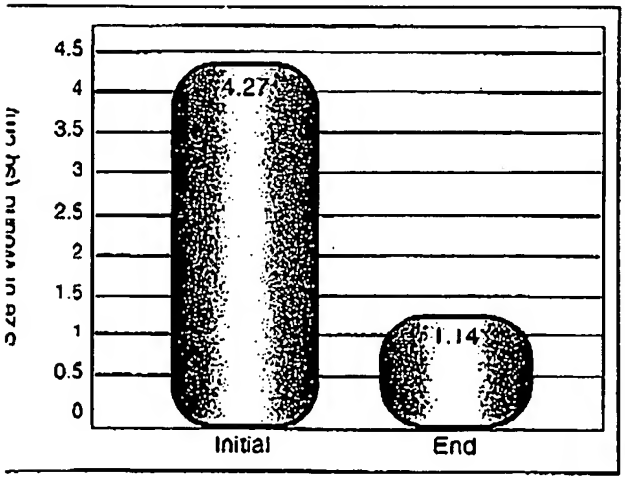
:Cell® was found to be highly effective on venous tasis ulcers in conjunction with compression therapy. ireater than 50% of the wounds healed within the ight-week study.

lealing data and average wound size are ummarized in Table 1 and Figure 3. One patient was ost to follow-up after Week 3 and the data for wound ize is not included in the graph of Figure 3. Six full ickness venous ulcers healed during the course of tudy, one at Week 2, two at Week 4, two at Week 6, ne at Week 7 (Figures 4 - 9). Duration of ulcer prior study initiation was long standing (range 1 month to reater than 12 months) and is documented in the ase Report Forms and Source Documentation.

able 1: **WOUND HEALING RESPONSE TO XCELL DRESSING ON VENOUS ULCERS (n=13)**

Ulcer Type	Healed	Improved
Partial Thickness	1	1
Full Thickness	6	5

gure 3: **Graph of the average decrease in wound size over time (wounds less than 50sq cm, n=12)**



Figures 4 - 6: Healing of a venous ulcer in a 79-year-old male presenting with a full thickness venous stasis ulcer on his left anterior lower leg. It was classified as a non-responsive wound that had lasted one to three months. Previous treatments included mechanical debridement and use of other wound dressings. Figure 4 demonstrates the ulcer after one week of XCell usage. Over the study, the wound continued to heal (Figures 5 & 6) to being fully healed at six weeks.

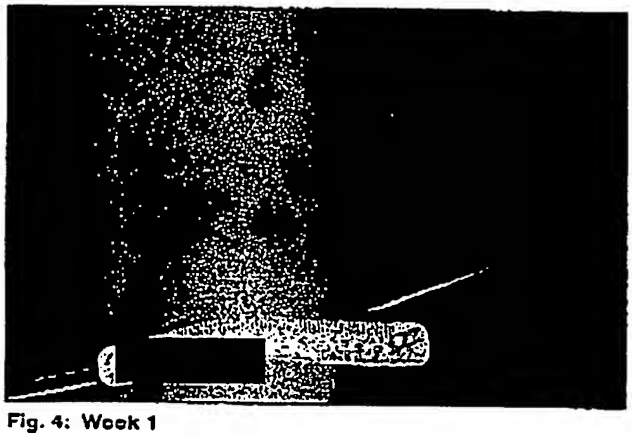


Fig. 4: Week 1

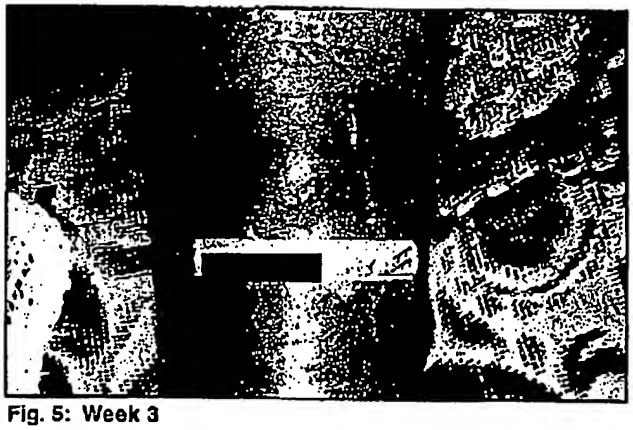


Fig. 5: Week 3

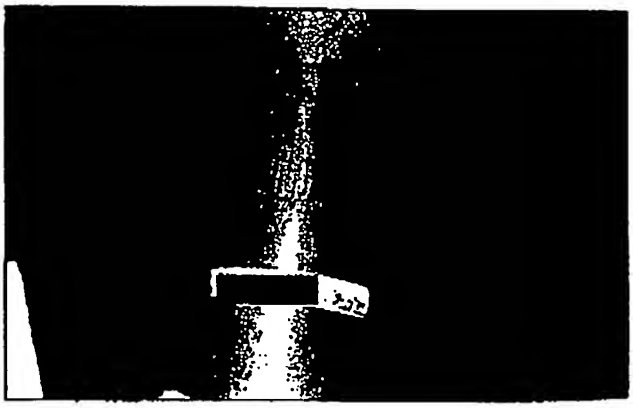


Fig. 6: Week 6

Figures 7 - 9: Healing of a venous stasis ulcer in 50-year-old female presenting with a venous stasis ulcer on her right lower foot. It was classified as a recurrent wound that had lasted over one year. It had 20% granulation tissue and 80% necrotic slough. Previous treatments included debriding, cleansing, exudate absorbing agents and protective coatings. Figure 7 demonstrates the ulcer at the initial visit. By Week 2 the slough had been debrided (Figure 8) and by Week 4 the ulcer had healed (Figure 9).

Fig. 7: Initial visit

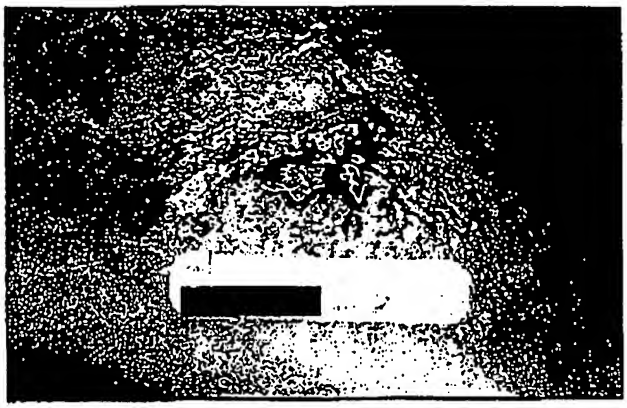


Fig. 8: Week 2

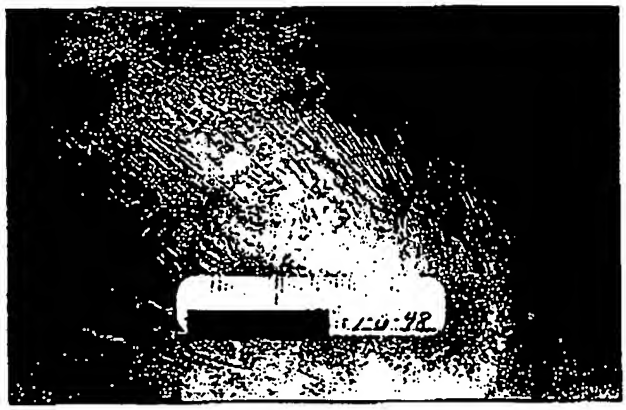


Fig. 9: Week 4

Figures 10 & 11: Healing using XCell® in a female presenting with a full thickness venous stasis ulcer on her left lateral lower leg. It was classified as a new wound that had lasted for over one year. Previous treatments included cleansing and exudate absorption agents and use of other wound dressings. Figure 10 shows the wound already healing at Week 2 with XCell. By Week 4 (Figure 11) the wound was fully healed.

Fig. 10: Week 2

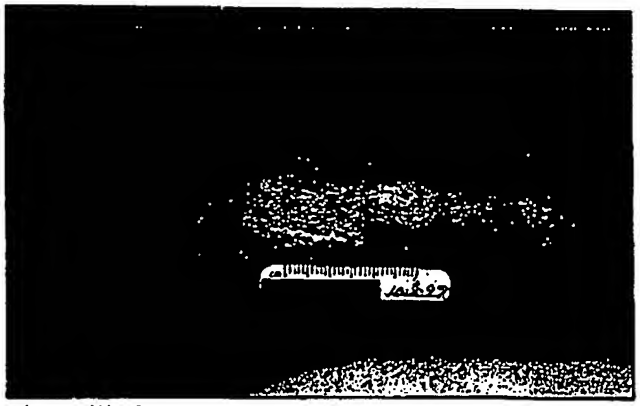


Fig. 11: Week 4

'enous Stasis Ulcer Evaluation Case Series of 13 Wounds

ive (5) full thickness venous ulcers demonstrated ıprovement with use of the cellulose wound ·cssing. Of note, one patient requested to remain on ıis dressing at time of study conclusion because of ıe elimination of pain at the ulcer site. Of the two ırtial thickness ulcers one healed and the other emonstrated improvement over the eight-week eatment (Figures 12 - 15).

gures 12 - 15: Improvement of a partial thickness venous asis ulcer in a 75-year-old male. It was classified as a non-sponsive wound that had lasted four to six months. It was ırtial thickness with 95% granulation tissue and 5% ıcrotic slough. Previous treatments included mechanical ıd chemical debridement and use of other wound essings. Figure 12 shows the state of the leg at the initial sit. By Week 3 (Figures 13 & 14) the ulcer had improved and ough was visible on the dressing. Note that the dressing ıd dried at periwound. The improvement continued to the ıd of the study at eight weeks (Figure 15).

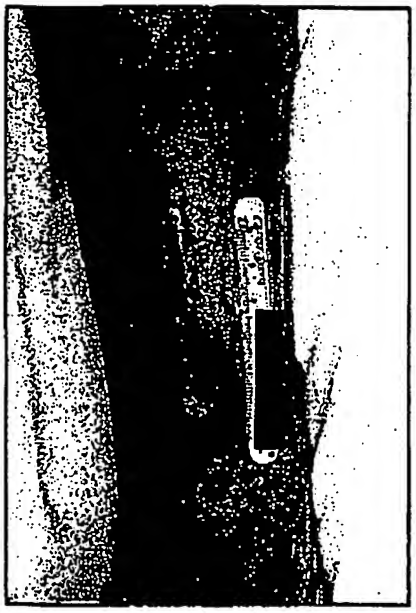


Fig. 12: Initial visit

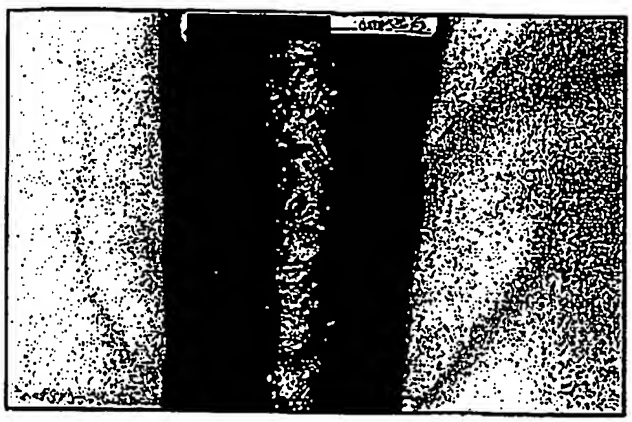


Fig. 13: Week 3

Fig. 14: Week 3 (Removal of dressing)

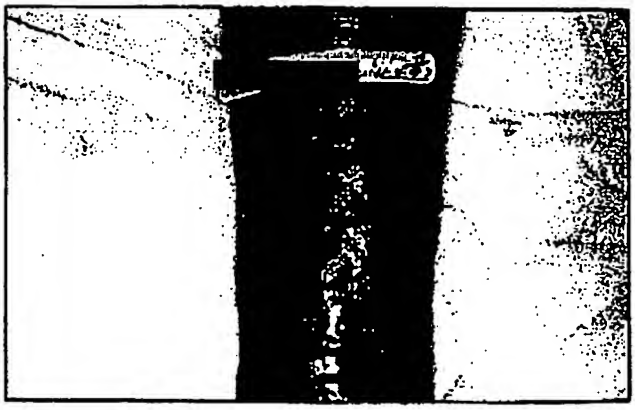


Fig. 15: Week 8

SUMMARY

XCell® Cellulose Wound Dressing performed well in the healing of venous ulcers. The most significant wound healing response noted with the use of XCell was the complete healing in full thickness venous leg ulcers. It should also be noted that during the course of the study, compression therapy was maintained from initial visit throughout the treatment duration for venous ulcers and, in most cases, the participant had been using that same form of compression prior to the initial visit.

The suggested treatment for venous stasis ulcers using XCell is to apply the dressing underneath an Unna's boot that is then wrapped with a compression bandage (for instance Coban™, 3M, Minneapolis, MN). XCell can be left in place for up to seven days depending on the level of exudate.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Gonzalo SERAFICA et al.

Title: MICROBIAL CELLULOSE WOUND DRESSING FOR TREATING CHRONIC WOUNDS

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DECLARATION UNDER 37 C.F.R. 1.132 OF CHRISTOPHER J. DAMIEN, Ph.D.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The undersigned, Christopher J. Damien, Ph.D., does hereby declare and state that:

1. I make the following declaration based upon my knowledge and belief and supporting exhibits and experiments.

2. I received my Ph.D. in Biomedical Engineering from Rutgers University and the University of Medicine and Dentistry of New Jersey. I am currently the Director of Research and Development at Xylos, where I oversee research and development relating to microbial cellulose biomaterials for use in medical applications.

3. I have reviewed the Office Action mailed on Aug. 15, 2006, as well as the cited U.S. Patent Nos. 4,588,400 and 6,320,093 to Ring and Augustine, respectively (hereafter "Ring" and "Augustine") and the Krystynowicz publication.

4. In my opinion, based on the facts and evidence described below, Ring, Augustine, and Krystynowicz, whether considered individually or in combination, do not teach or suggest our claimed invention.

5. To test Examples 4, 7, and 8 of Ring, which were cited in the Office Action, I supervised the following set of experiments to test liquid donation capacity. Materials were prepared by adding glycerol ('400 Example 4), polyethylene glycol (PEG) ('400 Example 4), silver sulfadiazine (SSD) ('400 Example 7), and petrolatum ('400 Example 8) to microbial cellulose according to the amounts and under conditions described in those examples:

I. Introduction

U.S. Patent # 4,588,400 to Ring describes a cellulose pad that can **either** supply moisture to wound site **or** absorb exudate generated by the wound [column 3 lines 28-30]. According to Example 1 of the '400 patent Ring prepared saturated pellicles that contained cellulose at approximately 40 g/M^2 and had a water content of 3600 g/M^2. Processing described in Examples 1 and 2 resulted in a range of cellulose pads with a ratio of liquid to cellulose of 2:1 to 20:1 or effectively 33.3% to 4.76% cellulose. The following is taken directly from the Ring '400 patent:

> *"EXAMPLE 2*
> *A water loaded pellicle prepared according to Example 1 was hand-pressed between absorbent sheets to reduce the water content to about 320 g/M^2 and to compress the pellicle into a thin, strong, wet, membrane-like sheet. The membrane had a thickness of less than about 1 mm and the weight ratio of liquid to cellulose in the membrane was approximately 8:1. Membranes having a weight ratio of liquid to cellulose in the range of from about 2:1 to 20:1 may be prepared in a similar manner. The compressed material is suitable for use as a protective wound covering or surgical wipe. When applied to wounds and covered with an occulsive* (sic) *backing film, such membranes have a capacity to absorb large quantities of wound exudate. "*

Additional Examples describe soaking a 8:1 ratio pad in glycerol or polyethylene glycol (PEG) to a ratio of 50:1 (Example 4); in 1% silver sulfadiazine (SSD) ointment to a ratio of 25:1 (Example 7); or in water to a ratio of 50:1 followed by a soak in melted petrolatum (Example 8). These suggest a cellulose content of 2%, 3.8% and 2%, respectively.

This experiment focused on preparing materials as described in Ring '400 Examples 2, 4, 7 and 8 and comparing their absorption and donation capabilities to those of Xylos's microbial cellulose wound dressing.

II. Equipment/Materials

a. 10 Processed microbial cellulose pellicles (lot # SA112205)
b. Hydraulic press (PP01) and shims
c. Plastic trays with lids, sufficient size and shape for testing
d. Analytical Top loading balance (B05)
e. 2-inch diameter arch punch
f. Punch cutting pad
g. Non-marring mallet
h. 3-4 oz Natural Import Strap Side leather
i. NaCl – isotonic saline (0.9%)
j. Synthetic Foam Sponges
k. Filtered, deionized water
l. Plastic film (PET, 2mil, Tekra, New Berlin, WI)
m. Materials:
    - Petrolatum: Vaseline (Chesebrough-Ponds USA, Greenwich, CT); Lot 02203H00
    - Silver sulfadiazine 1% (SSD): NDC 0591-0810-55 (Watson Laboratories, Inc., Corona CA)
    - Glycerol: G-7757 Lot 10K0185 (Sigma Chemical Co, St. Louis, MO)
    - Polyethylene glycol MW 400 (PEG): AC19223-0010 (Fisher Scientific Co, LLC, Pittsburgh, PA)

Table 1: Number of samples and tested

Material Tested	Absorption	Donation
Xylos Wound Dressing	3	3
50:1 Xylos Control	3	3
25:1 Xylos Control	3	3
Glycerol	3	3
PEG	3	3
SSD	3	3
Petrolatum	3	3

III. Procedures

a. Initial cellulose content

- Three cleaned pellicles were obtained and numbered 1 – 3
- All three were pressed as per Xylos SOPs for preparation of standard wound dressing using a 2.4 shim for 30 seconds to a weight of 100 ± 15g. Weights (**A**) were 114.2g, 97.3g and 93.9g.
- One 2" diameter circle was punched from each pellicle, weighed (**B**) and dried overnight.
- The dry samples were weighed (**C**) and the percent cellulose (**D**) and initial g cellulose in the pellicles (**E**) were determined.

Eq 1: **D = C / B**	**3.39 ± 0.16 % cellulose**
Eq 2: **E = D * A**	**3.44 ± 0.24 g cellulose (pellicle)**

b. Experimental pellicle setup

- Seven (7) additional cleaned pellicles were obtained and numbered 4 – 10.
- The weight of a pellicle needed to achieve 11.1% cellulose (**F**) was calculated by taking the average of **E** (above) from the three initial samples.
  Eq 3: **F** = [average (**E**)] / 11.1% **31.0 g for 11.1% pellicles**
- Pellicles 4 – 10 were pressed twice using no shims for 120 seconds to a weight of **F** (± 15%**F**)
- One 2" diameter circle from each pellicle was punched, weighed and dried overnight. Samples were reweighed the next day and the cellulose % was determined using Eq 1.
- Three pellicles with the ratio of water to cellulose that were closest to 8:1 (11.1% cellulose content) were chosen and included pellicles 5, 6, and 7. **(10.6%, 10.8% and 11.2% respectively).**
- Twelve 2" circles were punched from each of the three chosen pellicles. The remaining four pellicles and the skeletons of the punched pellicles were discarded.

c. Rehydrated Control sample prep

Three sets of controls were prepared for this study; one from the ~100g pellicles and two from the 11.1% pellicles.

- Xylos Wound Dressing: From the three pellicles originally weighing ~100g, two 2" circles were punched from each to act as wound dressing controls for absorption and donation testing.
- 50:1 Xylos Control: Six of the samples from the 11.1% pellicles (two per pellicle) were soaked in filtered water until they reached 5.67±0.01X their initial weight.
- 25:1 Xylos Control: Six of the samples from the 11.1% pellicles (two per pellicle) were soaked in filtered water until they reached 2.88±0.00X their initial weight.

d. Glycerol sample prep

Two circles from each of the three pellicles that were originally dehydrated to 11.1% cellulose were soaked in glycerol until the samples weighed approximately 5.67X their initial weight.

Actual weight was 5.42 ± 0.29X.

e. PEG sample prep

Two circles from each of the three pellicles that were originally dehydrated to 11.1% cellulose were to be soaked in polyethylene glycol (PEG – MW 400) until the samples reached approximately 5.67X their initial weight.

This was not possible, in that the samples only gained 3.35 ± 0.24X their weight after being soaked for >72 hours.

f. SSD sample prep

SSD cream was melted and two circles from each of the three pellicles that were originally dehydrated to 11.1% cellulose were soaked in the melted SSD cream until the samples weighed approximately 2.89X their initial weight.

Actual weight was 2.36 ± 2.3X.

g. Petrolatum sample prep

Two circles from each of the three pellicles that were originally dehydrated to 11.1% cellulose were soaked in water until the samples weighed approximately 5.67X their initial weight.

Actual weight was 5.68 ± 0.03X. They were then immersed in melted petrolatum at 100°C for 1 hour, removed and allowed to drain for 10 minutes.

h. Absorption Testing

Following Xylos SOP QD104 – "Determination of Fluid Absorbency of Wound Dressings or Gel" samples from each process were tested for their absorbency per Table 1. Briefly samples were weighed and placed onto a standard sponge that was immersed in normal saline to the top of the sponge. The container was closed and the sample was allowed to absorb saline for 24 hours. After, the sample was reweighed and the amount of absorption was calculated and expressed as a percent of the initial weight.

i. Donation Testing

Following Xylos SOP QD103 – "Determination of Fluid Donation of Wound Dressings or Gels" samples from each process were tested for their donation per Table 1. Briefly samples were weighed and placed onto a piece of leather that had also been weighed. The leather acts as a surrogate to skin, being cowhide. The samples were covered with PET to ensure that moisture was not released into the air. After two hours the leather was reweighed and the amount of liquid donated from the sample to the leather calculated and expressed as a percent of the initial weight.

IV. Results and Discussion

Cellulose Content of Xylos pellicles

Pellicles used in Xylos's wound dressing had a cellulose content of 50.7 ± 8.5 g/M^2 cellulose which was about 25% greater than the 40g/M^2 that was described in Example 1 of Ring '400.

Three sets of controls were prepared at the following liquid to cellulose ratios:

	Target Ratio	Actual Ratio (% cellulose)
1) Xylos Wound Dressing		28.5:1 (3.39%)
2) 50:1 Xylos Control	50:1	47.6:1 (2.06%)
3) 25:1 Xylos Control	25:1	24.1:1 (3.98%).

Glycerol and polyethylene glycol (MW 400)

In Example 4 of Patent '400, Ring describes the preparation of samples as follows:

" EXAMPLE 4
The method of Example 2 was repeated except the membrane-like sheet material was partially reconstituted with glycerol in one case and with polyethylene glycol (MW 400) in a second case. The resulting product in each case contained about 2000 g/M^2 liquid, was strong and flexible with good handle and drape, and did not dry out when exposed to air. Each of the samples was transparent to an extent permitting visual examination of skin condition through the dressing. The material was substantially lint-free and was suitable for use as a general purpose dressing. "

To compare Xylos microbial cellulose to that found in Example 4 of Ring '400 three pellicles were prepared to 11.1% cellulose or a ratio of 8:1 per method of Example 2. Test samples were then punched and partially reconstituted with glycerol and polyethylene glycol (MW 400) to match the ratio found in the example (2000 g/M^2 liquid to 40g/M^2 cellulose or 50:1). The samples absorbed glycerol until they weighed 5.42 ± 0.29X their initial weight or a liquid to cellulose ratio of 45:1 (cellulose content 2.27%).

It was more difficult to recreate Ring's ratio of liquid to cellulose when it was soaked in PEG. After 72 hours the samples only weighed 3.35 ± 0.24X their initial weight translating to a ratio of liquid to cellulose of 28:1 (rather than 50:1). Further testing was performed at this ratio, a cellulose content of 3.45%, which was similar to the Xylos Wound Dressing control at 3.39%.

Figure 1 illustrates the amount of liquid donated comparing Xylos controls to Ring's Example 4 at comparable liquid to cellulose ratios. Xylos controls donated significantly more liquid ($p<0.001$; t-Test) than both '400 Example 4 materials.

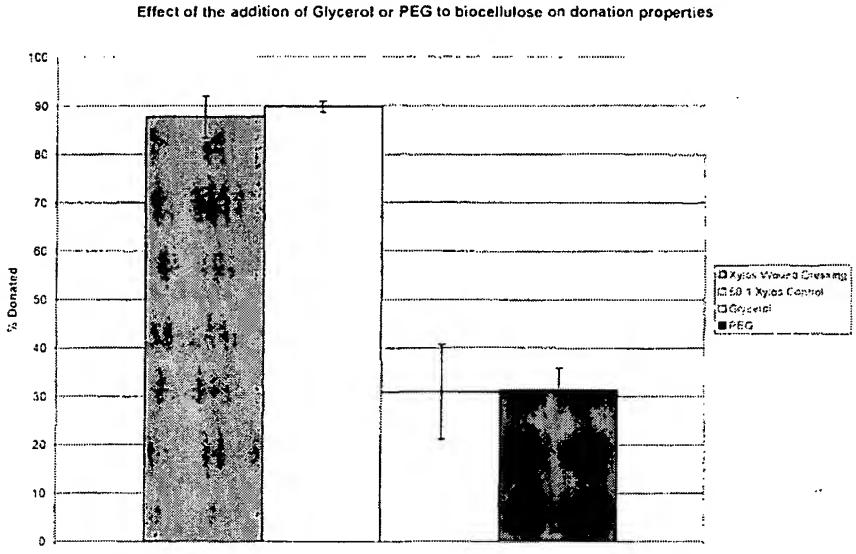
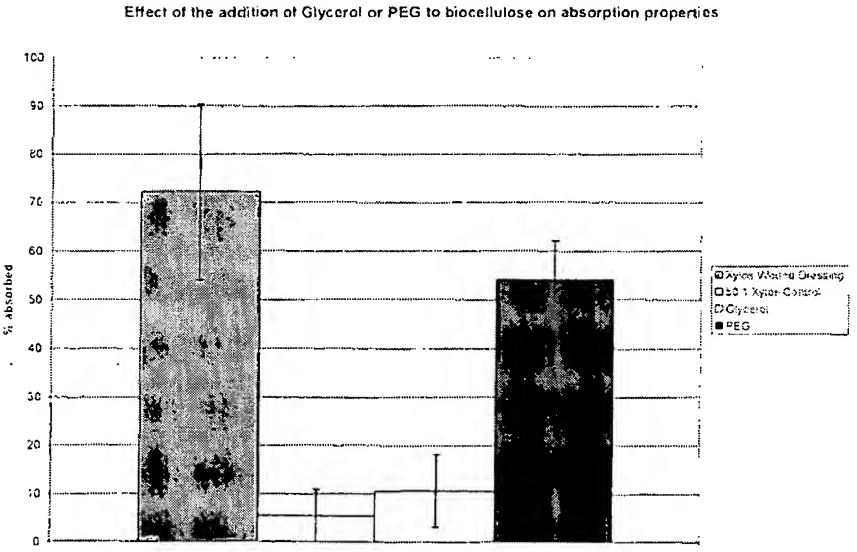


Figure 2 illustrates the absorption of liquid comparing Xylos controls to '400 Example 4 materials. Comparing similar cellulose contents (Xylos Wound Dressing to PEG and 50:1 Xylos Control to glycerol) there were no statistical differences in absorption.



Discussion
Xylos Wound Dressing control with 3.39% cellulose demonstrated greater than 80% donation and 70% absorption. This can be compared to Ring's PEG sample at 3.45% cellulose that donated significantly less liquid (~31%) but absorbed a similar amount. The 50:1 Xylos control demonstrated significantly more donation with a 90% donation rate compared to only 30% for the glycerol. They had similar absorption amounts.

Conclusion: Although the ratios of liquid to cellulose were similar, addition of glycerol or PEG (MW 400) resulted in a statistically significant decrease in the available moisture that was donated compared to Xylos material. This did not affect the absorption characteristics, which were similar to the Xylos Controls. This demonstrates that material with a liquid to cellulose ratio of 28:1 described in the '400 patent is different from Xylos's wound dressing at a 28.5:1 ratio.

Silver sulfadiazine (SSD)
In Example 7 of '400, Ring describes the preparation of samples as follows:

"EXAMPLE 7
The method of Example 2 was repeated except the membrane-like sheet was reconstituted with 1% silver sulfadiazine (SSD) ointment. The compressed pellicle was immersed in SSD ointment which had been warmed to fluid state until the liquid content of the pellicle had increased to about 1000 g/M^2. The impregnation with SSD ointment was conducted in a darkroom and the resulting product was packaged in a light proof, moisture-impervious aluminum foil packet and was suitable for use as a burn dressing. A similar product is obtained by impregnating the membrane-like sheet with an aqueous solution of zinc sulfadiazine. In another embodiment, the membrane-like sheet may be surface-coated with silver sulfadiazine powder so that the antiseptic is applied directly to the burn site."

To compare Xylos microbial cellulose to that found in Example 7 of Ring '400 three pellicles were prepared to 11.1% cellulose or a ratio of 8:1 per method of Example 2. Test samples were then punched and reconstituted with 1% SSD ointment warmed to a fluid state to match the ratio found in the example (1000 g/M^2 liquid to 40g/M^2 cellulose or 25:1). The samples absorbed SSD until they weighed 2.36 ± 0.23X their initial weight or a liquid to cellulose ratio of 19:1 (cellulose content 4.90%) and then the absorption slowed such that 25:1 could not be obtained. Samples were tested at the 19:1 ratio.

Figure 3 demonstrates the significant differences ($p<0.0001$) in liquid donation of the Xylos material compared to that in Patent '400 Example 7.

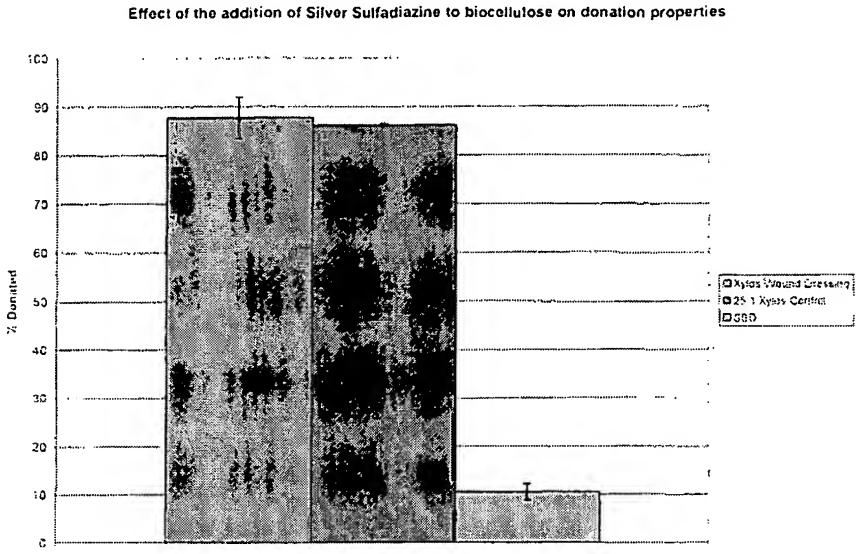
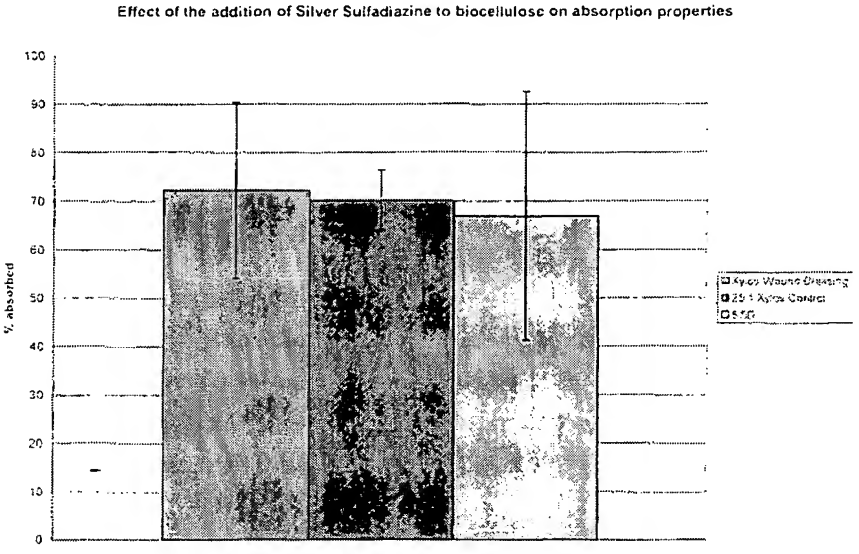


Figure 4 demonstrates the similar absorption rate of the Xylos material compared to that in Patent '400 Example 7.



Discussion:

The donation and absorption profiles in Figures 3 and 4 illustrate that the material in Ring '400 Example 7 clearly has only one function, that of absorption, at a liquid to cellulose ratio of 19.4:1 (4.9% cellulose). Xylos material at a decreased

cellulose concentration of 24.1:1(3.98%) and 28.5:1 (3.39%) demonstrated the dual functionality of absorption and donation at comparable levels.

Conclusion: Although the ratio of liquid to cellulose is lower in the Ring '400 material, addition of SSD resulted in a decrease in the available moisture that was donated and therefore demonstrates that these materials have different intrinsic properties.

Petrolatum

In Example 8 of '400, Ring describes the preparation of samples as follows:

"EXAMPLE 8
The method of Example 2 was repeated except the membrane-like sheet material was partially reconstituted with water to a loading of 2000 g/M^2. The water-loaded pellicle was immersed in melted petrolatum at a temperature of 100° C. for 1 hour. The pellicle was thereupon removed and allowed to drain. The resulting product was a petrolatum-coated dressing having a water core and had a reduced tendency to adhere to wounds."

To compare Xylos microbial cellulose to that found in Example 8 of Ring '400 three pellicles were prepared to 11.1% cellulose or a ratio of 8:1 per method of Example 2. Test samples were then punched and partially reconstituted with water to match the ratio found in the example (2000 g/M^2 liquid to 40g/M^2 cellulose or 50:1). The samples absorbed water until they weighed 5.68 ± 0.03X their initial weight or a liquid to cellulose ratio of 49:1 (cellulose content 2.0%). The materials were then further processed in the heated petrolatum as described in the procedure above. After one hour materials were removed drained and tested.

Figure 5 illustrates the statistically significant increase ($p<0.005$) in donation of liquid comparing Xylos to that in Ring '400 Example 8 at similar liquid to cellulose ratios of water.

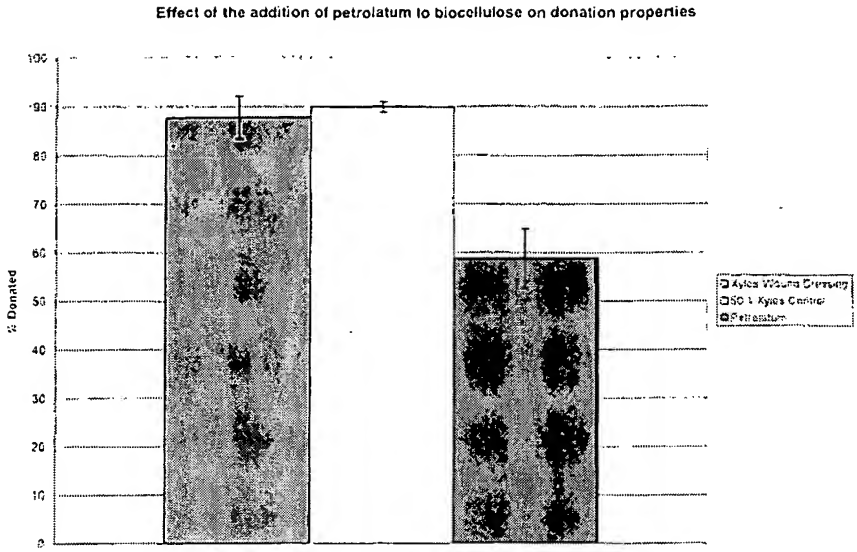
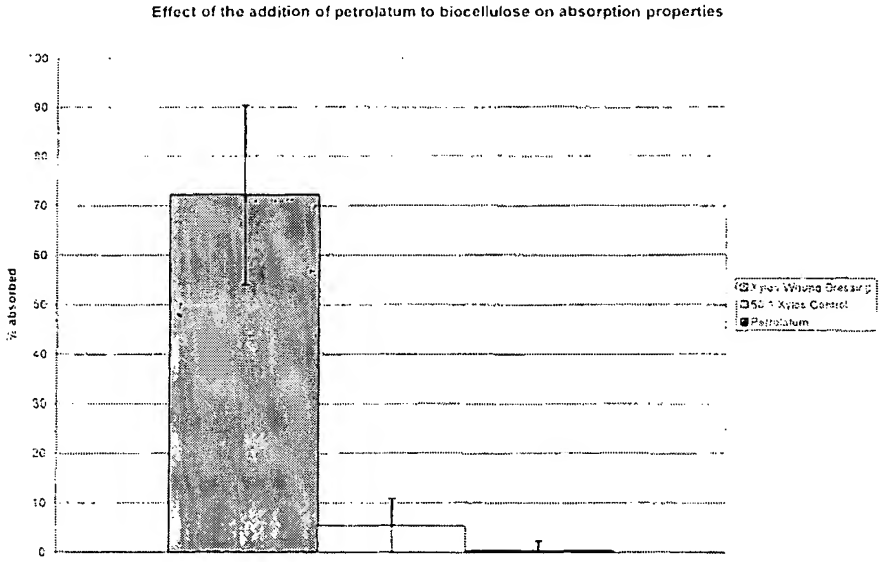


Figure 6 demonstrates the amount of liquid absorption of the Xylos material compared to the Ring '400 Example 8. The Xylos Wound Dressing control demonstrated significantly more absorption than the other two materials ($p<0.005$).



Discussion:

Material prepared with an excess of water should demonstrate donation but possibly less absorptive capacity. This is noted in the Xylos material at 47.6:1 versus 28.5:1 liquid to cellulose. Both demonstrate similar donation percentages while demonstrating different absorption ability. The material from Example 8 of

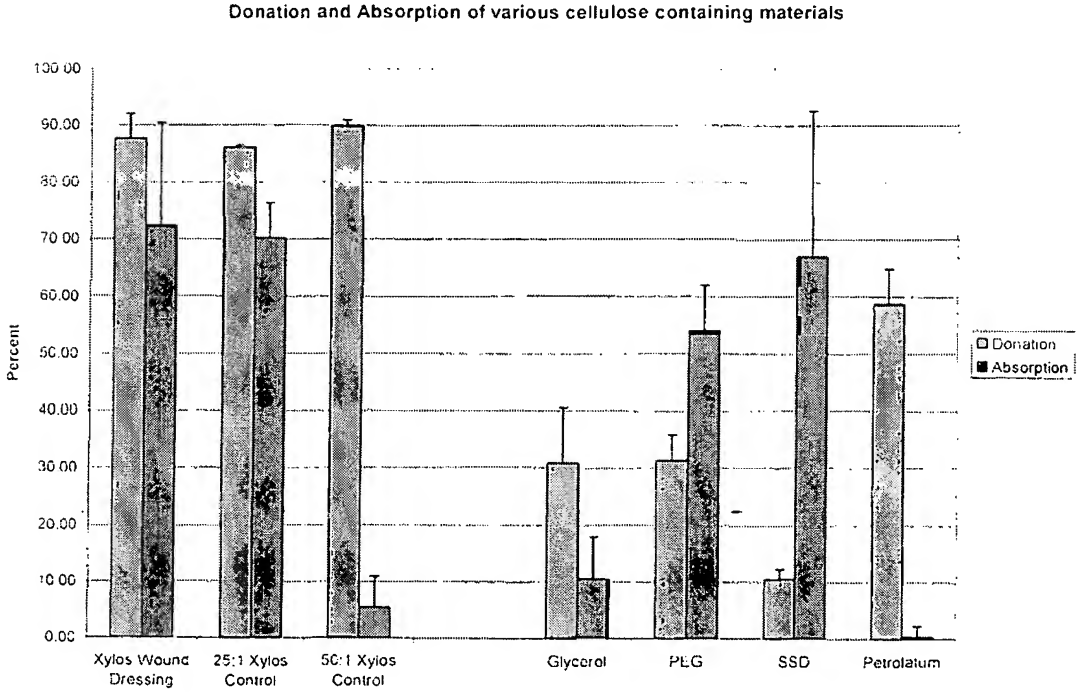
Ring '400, with a liquid to cellulose ratio of 49:1, shows a similar lack of absorptive capability and also significantly less ability to donate the moisture that is in the system.

Conclusion: Although the ratio of liquid to cellulose is similar, addition of petrolatum resulted in a significant decrease in the available moisture that could be donated to a wound as compared to Xylos material.

V. Overall Conclusions

Ring '400 describes a microbial cellulose pad with a liquid to cellulose ratio range of 2:1 to 20:1. Xylos Wound Dressings possess a range of liquid to cellulose ratio of 21:1 and greater and demonstrate the dual functionality of absorption and donation that is beneficial to healing chronic wounds. This dual action allows one dressing to both provide moisture and absorb wound fluid at the same time depending on the microenvironment of the wound on which the Xylos Wound Dressing is in contact. When testing materials prepared at similar liquid to cellulose ratios greater than the range stated in Ring '400 as described in the Examples, in no case is this dual functionality exhibited to the extent observed with Xylos material (Figure 7). Ring '400 describes a dressing that **either** provides moisture to the wound **or** absorbs exudate.

Figure 7: Summary data of all absorption and donation testing.



6. The above results demonstrate that Ring's examples 4, 7, and 8 fail to produce a dressing that could donate greater than 75% of its liquid weight to a chronic wound. This suggests that Ring did not envision a dressing with an increased liquid to cellulose ratio that could both donate and absorb simultaneously at levels sufficient to treat chronic wounds. In my opinion, since previous wound dressings have been designed for a single function it is surprising that a properly formulated microbial cellulose dressing can both donate and absorb sufficient amounts of liquid to treat chronic wounds.

7. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

9-20-06
Date

Christopher J. Damien, Ph.D.